EXHIBIT C-49

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National Toxicology Program
U.S. Department of Health and Human Services

Abstract for TR-598

Toxicology and Carcinogenesis Studies of Perfluorooctanoic Acid Administered in Feed to Sprague Dawley (Hsd:Sprague Dawley SD) Rats

CASRN: 335-67-1
Chemical Formula: C8HF15O2
Molecular Weight: 414.07
Synonyms/Common Names: PFOA
Report Date: May 2020

FULL REPORT PDF

ABSTRACT

Perfluorooctanoic acid (PFOA) is a perfluorinated alkyl substance (PFAS) with widespread exposure in the environment and human population. Lifetime exposure to this chemical is likely, which includes in utero and postnatal development. Previously conducted chronic carcinogenicity studies of PFOA began exposure after these critical periods of development, so it is unknown whether the carcinogenic response is altered if exposure during gestation and lactation is included. The current PFOA chronic studies were designed to assess the contribution of combined gestational and lactational exposure (herein referred to as perinatal exposure) to the chronic toxicity and carcinogenicity of PFOA. The hypothesis tested was that including exposure during gestation and lactation (perinatal exposure) with postweaning exposure would change the PFOA carcinogenic response quantitatively (more neoplasms) or qualitatively (different neoplasm types) compared to postweaning exposure alone.

This hypothesis was tested using a design of exposing time-mated Sprague Dawley (Hsd:Sprague Dawley SD) rats to 0, 150, or 300 ppm PFOA during the perinatal period, after which the F1 male rats were provided 150 or 300 ppm PFOA (i.e., perinatal/postweaning exposures of 0/0, 0/150, 150/150, 0/300, and 300/300 ppm) and the F1 female rats were provided 300 or 1,000 ppm PFOA (i.e., 0/0, 0/300, 150/300, 0/1,000, and 300/1,000 ppm) during the postweaning period (n = 50/sex/dose). Female

rats have a lower systemic exposure due to a faster PFOA elimination rate than males, so a higher feed exposure concentration was provided to female rats postweaning. An interim necropsy (n = 10/sex/group) at 16 weeks (19 weeks of age) was conducted.

Due to unanticipated toxicity in male rats observed at the 16-week interim time point, males were removed from the first study at week 21. A second study of males only was started that used lower postweaning feed concentrations. In this second study, the pregnant females were exposed to a single feed concentration of 300 ppm PFOA because this exposure was well tolerated.

Sixteen-week Interim Evaluation

In general, toxicity was observed in the liver, glandular stomach, kidney, and thyroid gland in males and in the liver, kidney, and thyroid gland in females at the 16-week interim evaluation. Body weights were lower in exposed groups of males and females compared to control groups as exposure concentrations increased. Plasma concentrations of PFOA were consistently higher in males compared to females and consistent between animals that were exposed to PFOA perinatally and postweaning versus postweaning exposure alone. Acyl-CoA oxidase activity in the liver was consistently elevated in males and females (males had higher activity than females) regardless of their exposure during the perinatal period.

Two-year Studies

Survival was unaffected by PFOA exposure, and there were exposure-related decreases in body weight compared to control groups in both male and female rats. Male rats had increased incidences of hepatocellular adenomas in the 0/40, 300/40, 0/80, and 300/80 ppm groups compared to the 0/0 ppm control group, and higher incidences of hepatocellular carcinomas were observed in the 300/80 ppm group compared to the 0/80 group. Increased pancreatic acinar cell adenomas and adenocarcinomas were observed in all postweaning exposed groups (20, 40, and 80 ppm) with or without perinatal exposure. Although not statistically significant, there were occurrences in female rats of pancreatic acinar cell adenomas and adenocarcinomas in the 0/1,000 and 300/1,000 ppm female groups compared to the 0/0 ppm control group. Marginally higher numbers of hepatocellular carcinomas and uterine adenocarcinomas were also observed in the PFOA-exposed groups regardless of perinatal exposure. Nonneoplastic lesions were only observed in the liver and pancreas of male rats, whereas lesions were increased in the liver, kidney, forestomach, and thyroid gland of female rats.

In general, very few significant differences were observed between the responses of groups of animals exposed to PFOA postweaning only versus groups with both perinatal and postweaning exposures, and most of these differences were considered sporadic. The response to PFOA in female rats was generally less than that of male rats, which was consistent with the lower internal plasma concentrations of PFOA in female rats relative to male rats.

Conclusions

Under the conditions of these 2-year feed studies, there was clear evidence of carcinogenic activity of PFOA in male Hsd:Sprague Dawley SD rats based on the increased incidence of hepatocellular neoplasms (predominately hepatocellular adenomas) and increased incidence of acinar cell neoplasms (predominately acinar cell adenomas) of the pancreas. The additional effect of perinatal exposure in combination with postnatal exposure was uncertain and limited to the observation of hepatocellular carcinomas.

There was some evidence of carcinogenic activity of PFOA in female Hsd:Sprague Dawley SD rats based on the increased incidences of pancreatic acinar cell adenoma or adenocarcinoma (combined) neoplasms. The higher incidence of hepatocellular carcinomas and adenocarcinomas of the uterus may have been related to exposure. The combined perinatal and postweaning exposure was not observed to change the neoplastic or nonneoplastic response compared to the postweaning exposure alone in female rats.

Exposure to PFOA resulted in increased incidences of nonneoplastic lesions in the liver and pancreas of male rats and in the liver, kidney, forestomach, and thyroid gland of female rats.

National Toxicology Program (NTP). 2020. NTP technical report on the toxicology and carcinogenesis studies of perfluorooctanoic acid (CASRN 335-67-1) administered in feed to Sprague Dawley (Hsd:Sprague Dawley SD) rats. Research Triangle Park, NC: National Toxicology Program. Technical Report 598. https://doi.org/10.22427/NTP-TR-598

STUDIES

Summary of the Two-year Toxicology and Carcinogenesis Studies of Perfluorooctanoic Acid with and without Perinatal Exposure

	Male Sprague Dawley Rats	Female Sprague Dawley Rats
Concentrations in feed		
Postweaning	0/0, 0/20, 0/40, 0/80 ppm	0/0, 0/300, 0/1,000 ppm
Perinatal + postweaning	300/0, 300/20, 300/40, 300/80 ppm	0/0, 150/300, 300/1,000 ppm
Survival rates		
Postweaning	36/50, 42/50, 34/50, 36/50	23/50, 26/50, 23/50
Perinatal + postweaning	34/50, 38/50, 38/50, 39/50	23/50, 32/50, 22/50
Body weights		

	Male Sprague Dawley Rats	Female Sprague Dawley Rats
Postweaning	0/80 ppm group: 82–90% of the 0/0 ppm control group weight after week 6	0/1,000 ppm group: 78–88% of the 0/0 ppm control group weight after week 2
Perinatal + postweaning	300/80 ppm group: 83–90% of the 0/0 ppm control group weight after week 6	300/1,000 ppm group: 73–86% of the 0/0 ppm control group weight after week 2
Nonneoplastic effects		
Postweaning	Liver: hepatocyte, cytoplasmic alteration (0/50, 12/50, 34/50, 46/50); hepatocyte, hypertrophy (0/50, 13/50, 34/50, 43/50); hepatocyte, single cell death (1/50, 1/50, 11/50, 24/50); necrosis (2/50, 17/50, 23/50, 20/50); pigment (0/50, 7/50, 15/50, 30/50) Pancreas: acinus, hyperplasia (18/50, 32/50, 37/50, 31/50)	Liver: hepatocyte, cytoplasmic alteration (0/50, 9/50, 49/49); hepatocyte, hypertrophy (0/50, 11/50, 48/49); hepatocyte, single cell death (0/50, 4/50, 29/49); necrosis (0/50, 1/50, 8/49); pigment (3/50, 5/50, 43/49); bile duct hyperplasia (16/50, 25/50, 22/49); hepatocyte, increased mitoses (2/50, 3/50, 4/49) Kidney: papilla, urothelium, hyperplasia (4/50, 21/50, 40/49); papilla, necrosis (0/50, 0/50, 12/49); renal tubule, mineral (5/50, 6/50, 16/49) Forestomach: ulcer (2/50, 2/50, 9/49); epithelium, hyperplasia (4/50, 5/50, 22/49); submucosa, inflammation, chronic active (3/50, 2/50, 16/49) Thyroid gland: follicular cell, hypertrophy (4/50, 8/50, 28/49)

	Male Sprague Dawley Rats	Female Sprague Dawley Rats
Perinatal + postweaning	Liver: hepatocyte, cytoplasmic alteration (0/50, 4/50, 29/50, 41/50); hepatocyte, hypertrophy (1/50, 4/50, 29/50, 42/50); hepatocyte, single cell death (1/50, 3/50, 5/50, 29/50); necrosis (1/50, 11/50, 14/50, 21/50); pigment (0/50, 4/50, 11/50, 26/50) Pancreas: acinus, hyperplasia (23/50, 27/50, 38/50, 33/50)	Liver: hepatocyte, cytoplasmic alteration (0/50, 17/50, 49/50); hepatocyte, hypertrophy (0/50, 16/50, 49/50); hepatocyte, single cell death (0/50, 5/50, 32/50); necrosis (0/50, 4/50, 5/50); pigment (3/50, 10/50, 40/50); bile duct hyperplasia (16/50, 27/50, 27/50); hepatocyte, increased mitoses (2/50, 5/50, 10/50) Kidney: papilla, urothelium, hyperplasia (4/50, 8/50, 45/50); papilla, necrosis (0/50, 0/50, 22/50); renal tubule, mineral (5/50, 8/50, 8/50) Forestomach: ulcer (2/50, 1/50, 11/50); epithelium, hyperplasia (4/50, 3/50, 21/50); submucosa, inflammation, chronic active (3/50, 2/50, 18/50) Thyroid gland: follicular cell, hypertrophy (4/50, 9/50, 19/50)
Neoplastic effects		

	Male Sprague Dawley Rats	Female Sprague Dawley Rats
Liver	Postweaning: hepatocellular adenoma (0/50, 0/50, 7/50, 11/50); hepatocellular carcinoma (0/50, 0/50, 0/50, 0/50); hepatocellular adenoma or carcinoma (0/50, 0/50, 7/50, 11/50) Perinatal + postweaning: hepatocellular adenoma (0/50, 1/50, 5/50, 10/50); hepatocellular carcinoma (0/50, 0/50, 0/50, 4/50); hepatocellular adenoma or carcinoma (0/50, 1/50, 5/50, 12/50)	None
Pancreas	Postweaning: acinar cell adenoma (3/50, 28/50, 26/50, 32/50); acinar cell adenocarcinoma (0/50, 3/50, 1/50, 3/50), acinar cell adenoma or adenocarcinoma (3/50, 29/50, 26/50, 32/50) Perinatal + postweaning: acinar cell adenoma (7/50, 18/50, 30/50, 30/50); acinar cell adenocarcinoma (0/50, 2/50, 1/50, 3/50), acinar cell adenoma or adenocarcinoma (7/50, 20/50, 30/50, 30/50)	Postweaning: acinar cell adenoma (0/50, 0/50, 1/49); acinar cell adenocarcinoma (0/50, 0/50, 1/49); acinar cell adenoma or adenocarcinoma (0/50, 0/50, 2/49) Perinatal + postweaning: acinar cell adenoma (0/50, 0/50, 3/50); acinar cell adenocarcinoma (0/50, 0/50, 2/50); acinar cell adenoma or adenocarcinoma (0/50, 0/50, 5/50)
Equivocal findings		

	Male **Sprague Dawley Rats**	**Female** **Sprague Dawley Rats**
Liver	None	Postweaning: hepatocellular carcinoma (1/50, 1/50, 3/49) Perinatal + postweaning: hepatocellular carcinoma (1/50, 0/50, 4/50)
Uterus	None	Postweaning: adenocarcinoma (1/50, 5/50, 8/50) Perinatal + postweaning: adenocarcinoma (1/50, 3/50, 5/50)
Level of evidence of carcinogenic activity	Clear evidence	Some evidence

EXHIBIT C-50

AR226-0639

3PP

PFOS Presentation to CMA, June 19, 2000
Charles M. Auer, Director
Chemical Control Division
U.S. Environmental Protection Agency

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BACKGROUND

PFOS (perfluorooctane sulfonic acid) is a member of a large family of sulfonated perfluoro-chemicals (total annual production of the PFOS family is < 10 million lbs) which are used for a wide variety of industrial, commercial, and consumer applications (including use as a component of soil and stain-resistant coatings for fabrics, leather, furniture, and carpets, in fire-fighting foams, commercial and consumer floor polishes, cleaning products, and as a surfactant in other specialty applications). Pesticidal and indirect food use products are also made from this technology.

All of these chemicals have the potential to degrade back to PFOS which does not appear to degrade further (it is thus highly persistent). 3M Corporation is the sole US manufacturer of the PFOS family of chemicals.

PFOS has been found widely in human blood samples (ppm levels in manufacturing workers, ppb levels in non-exposed workers and in blood bank samples) based on 3M research. PFOS has also been found in wildlife species across the US (especially in fish eating birds) and was detected in naive (unexposed) laboratory rats (the PFOS contamination was traced back to fish meal used in the rat chow).

PFOS caused postnatal deaths (and other developmental effects) in offspring in a 2-generation reproductive effects rat study (NOAEL of 0.1 mg/kg/day and LOAEL of 0.4 mg/kg/day). At higher doses in this study, progeny in the first generation died, while at the LOAEL reduced pup weight gain was observed.

PFOS accumulates to a high degree in humans and animals. It has an estimated half-life of 4 years in humans. It thus appears to combine Persistence, Bioaccumulation, and Toxicity properties to a high degree.

3M had previously launched a major research effort on PFOS to characterize its environmental presence, environmental and human effects, and environmental fate. EPA continues to receive the results of this work and will make it available as it comes in.

EPA REVIEW

Preliminary data indicated to EPA that PFOS is of significant concern on the basis of evidence of widespread human exposure and indications of toxicity in the 2-generation rat study. In addition, EPA's preliminary risk assessment indicated potentially unacceptable margins of exposure (MOEs) for workers and possibly the general population.

There are many assumptions and considerable uncertainty in these arguments and analyses. It is not possible at present to judge the adequacy or accuracy of the MOE analyses or whether the exposure levels used in the above estimations may be considered representative of the affected populations at large.

EPA has requested detailed information from 3M and a large body of information has been received but not fully reviewed. Review of subchronic studies provided by 3M on monkeys and rats also show deaths at doses similar to those reported in the 2-generation study.

3M has raised questions regarding the possible relevance to humans of a proposed mechanism (effects on cholesterol biosynthesis) for PFOS's lethal effect in the 2-generation study. The proposed mechanism, the company argues, affects reproductive outcomes in litter bearing animals due to its inhibitory effect on a burst of cholesterol biosynthesis in the critical period just before birth.

The proposed mechanism would, if demonstrated, have broad implications for and present significant potential concerns for humans and environmental organisms. At this time it is not clear what is going on in PFOS toxicology – nonetheless, the persistent presence of deaths in multiple studies involving multiple species at roughly equivalent doses raises flags.

PHASE OUT DECISION BY 3M

Following a series of discussions with EPA, and based on concerns about the widespread presence and longer term risks presented by PFOS, 3M announced that it would exit worldwide from this market by about the end of the year, although it may need to extend the time period for some critical uses (e.g., fire fighting foam).

EPA agrees that continued manufacture and use of PFOS represents an unacceptable technology that should be eliminated to protect human health and the environment from potentially severe long term consequences. The company has committed to continue its research effort despite the commercial decision. 3M has expressed interest in collaborative efforts with EPA as they withdraw from the market and in the development of safer substitutes.

EPA is currently examining appropriate regulatory alternatives necessary to protect human health and environment in light of 3M's phaseout decision on PFOS. More information will be made available as our strategy becomes clarified.

EPA strongly supports continued research on PFOS to improve our understanding of it fate and effects to humans and the environment. A more complete understanding of the environmental fate of PFOS derivatives, including polymers, is particularly important to allow an assessment of the longer term consequences of the PFOS which has been released into the environment.

EPA is prepared to work with industry, both manufacturers and users, to assist in review of critical uses of PFOS to ensure that good decisions are made in those cases where risk/risk

tradeoff issues are presented by the phaseout decision. These uses include fire fighting foam and acid mist suppression.

PFOS ALTERNATIVES AND RELATED SUBSTANCES

Users of PFOS-based products are confronting the need to replace PFOS. Various materials have been identified as substitutes for these uses. EPA recommends that alternatives be carefully evaluated to identify possible hazard or risk issues. EPA is prepared to work with individual manufacturers of alternatives which are available or under development as substitutes for PFOS to ensure that good decisions are made.

As the work on PFOS progresses, EPA plans to broaden its review to encompass other highly fluorinated acids, including PFOA and other materials, including the telomers.

PFOA

PFOA (perfluorooctanoic acid) is closely related structurally to PFOS and is used as a solvent for certain polymerization reactions. EPA has requested information from producers and will be preparing an assessment. Based on preliminary information, PFOA presents a different hazard, exposure, and risk picture compared to PFOS. 3M has also committed to ending production of PFOA. There are other producers in the US and EPA is examining its options regarding action on PFOA. We are aware of industry concerns regarding the availability of substitutes for PFOA in its fluoropolymer reaction solvent application.

TELOMERS AND OTHER PERFLUORO CHEMISTRIES

EPA has not yet looked into the telomers and other perfluoro chemistries. The ITC will be requesting information on a broad array of fluorinated derivatives and we will be working with them as part of our assessment effort. The telomers represent an interesting alternative to PFOS and we encourage industry efforts to inform EPA and others regarding the fate and effects of these materials. Of particular interest to EPA in this regard is to understand the environmental fate of these materials, including to what extent do the various derivatives degrade to PFOA and what are the rates and extent of this degradation under various environmental conditions.

EXHIBIT C-51

3M Confidential -- FC Issue

Attorney Client and Work Product Privileged
Do Not Copy - Do Not Disclose
3M Proprietary Information

Concise "Plain English" Key Message Talking Points

Draft -- 7/13/99

For use in verbal communications such as TV interview with general, non-scientific audiences:

Primary

- New techniques, developed by 3M scientists, enable us to track materials at trace levels previously impossible to detect or identify in the human body and the environment
- The discovery indicates no harm to people, wildlife or the environment
- There are no health effects -- no one is ill
- We've shared what we know with government officials (regulators), customers and even competitors in our industry
- We're working with independent experts to further scientific understanding
- This research will help advance the fields of health, safety and environmental protection
- We've used this technology to refine and reformulate (find alternative ingredients for) 3M products, such as fire-fighting foams and medical supplies

Secondary

- We make safe products -- products that people want and need
- We've been looking at this for more than 30 years
- Our factory and laboratory employees who work with this chemistry on a daily basis (and who have levels 100 times higher than general samples) show no signs of any health effects
- We provide safe workplaces for our employees
- 3M is doing the right thing... in keeping with our tradition, culture and values
- We're always doing more to improve our products and processes
- Nobody knows more about the science of fluorine chemistry than 3M
- We're publishing articles about this breakthrough discovery in the scientific literature
- We will continue to share information with anyone who wants to know more

Attorney Client and Work Product Privileged
Do Not Disclose

EXHIBIT C-52

MAY. 16. 2000 10:04AM DUPONT INVESTOR RELATIONS NO. 4109 P. 1/3

DUPONT
The miracles of science

May 16, 2000

Via Facsimile
Chad Holliday - 30737
Richard Goodmanson - 34244
George MacCormack – 22767
Paul Tebo – 41361
Tom Connelly – 67156
Don Johnson – 22645

3M Announcement

Attached is forwarded for your information.

DBM
D. B. MILLER

Attachment

DGW 000038

EID166245

FOR IMMEDIATE RELEASE

Investor Contact: Jon Greer
651-736-1915

Media Contact: John Cornwell
651-733-7698

3M Phasing Out Some of its Specialty Materials

ST. PAUL, Minn. – May 16, 2000 – 3M today announced it is phasing out of the perfluorooctanyl chemistry used to produce certain repellents and surfactant products.

The affected product lines represent about two percent of 3M's nearly $16 billion in annual sales. These include many Scotchgard™ products, such as soil, oil and water repellent products; coatings used for oil and grease resistance on paper packaging; fire-fighting foams; and specialty components for other products. 3M said it plans to substantially phase out production by the end of the year and will work with customers to accomplish a smooth transition.

"Our decision anticipates increasing attention to the appropriate use and management of persistent materials," said Dr. Charles Reich, executive vice president, Specialty Material Markets. "While this chemistry has been used effectively for more than 40 years and our products are safe, our decision to phase out production is based on our principles of responsible environmental management."

"We're reallocating resources to accelerate innovation in more sustainable opportunities and technologies. This decision is not only in the public interest, it's in the best interests of all our constituencies ... our employees, customers, communities and investors," Reich said.

Sophisticated testing capabilities – some developed in only the last few years – show that this persistent compound, like other materials in the environment, can be detected broadly at extremely low levels in the environment and in people. All existing scientific knowledge indicates that the presence of these materials at these very low levels does not pose a human health or environmental risk.

DGW 000039

Post-it® Fax Note 7671 | Date 5/16 | # of pages ▶ 2
To DAVE MILLER | From DAVE POWELL
Co./Dept. | Co. 3M
Phone # | Phone # EID166246
Fax # 302/773-2637 | Fax #

About 1,500 out of 3M's global work force of 71,000 employees have jobs associated with these products. "Innovation at 3M is at an all-time high, and there are many great opportunities for employees across the company," Reich said.

3M expects to meet consensus earnings estimates for the rest of 2000. This excludes a one-time charge on the order of $200 million, that will be taken sometime this year.

"Our growth engines are more powerful than ever and we're confident in our ability to continue delivering on expectations," said L.D. DeSimone, chairman and CEO. "Many of our new technology platforms directly address the fastest-growing segments of the new economy such as electronics, telecommunications and flat-panel displays."

"We expect the positive momentum in our financial performance to continue into 2001 with earnings somewhat above current analyst estimates," DeSimone said.

3M is a leading manufacturer of innovative products for industrial, consumer, transportation, safety, health care and other markets, with operations in more than 60 countries worldwide. The company is well known for its "Pollution Prevention Pays" environmental initiative, and its emission reduction programs including water-based replacement of solvents in manufacturing and replacements for ozone-depleting chlorofluorocarbons (CFCs).

Forward-Looking Statements

Certain portions of this news release that do not relate to historical financial information constitute forward-looking statements. These forward-looking statements are subject to certain risks and uncertainties. Actual future results and trends may differ materially from historical results or those expected depending on a variety of factors, including: (1) worldwide economic conditions; (2) foreign exchange rates and fluctuations in those rates; (3) the timing and acceptance of new product offerings; (4) raw materials, including shortages and increases in the costs of key raw materials; and (5) legal proceedings.

- # # # -

FROM: 3M Public Relations
3M Center, Building 225-1S-15
St. Paul, MN 55144-1000
651-733-8805
www.3m.com

DGW 000040

EXHIBIT C-53

DuPont Haskell Laboratory
for Toxicology and Industrial Medicine
Elkton Road, P. O. Box 50
Newark, DE 19714-0050

DuPont Haskell Laboratory

Gerald L. Kennedy
Director, Applied Toxicology
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VIA FEDERAL EXPRESS

Document Control Office (7407)
Room G99 East Tower ATTN: FYI
Office of Pollution Prevention and Toxics
U.S. Environmental Protection Agency
401 M Street, S.W.
Washington, D.C. 20460-0001

March 8, 2001

Dear Sir/Madam:

FYI-0101-001378

This letter is in reply to Richard H. Hefter's letter dated February 14, 2001 to me concerning the above-referenced FYI submission.

The information provided in the January 25, 2001 and June 23, 2000 submissions was provided voluntarily to EPA in response to a request from Mr. Charles Auer for exposure and use information from DuPont pertaining to the substance in issue. The blood serum test results reflect exposure data. No adverse health effects associated with this substance have been found among exposed employees. It is further noted that studies submitted by the 3M Company, which is the manufacturer of the substance, also conclude that no health effects associated with this substance have been found amongst its exposed employees (see 3M 8EHQ-0200-14596, dated January 28, 2000). Those studies, either themselves or through reference to other studies published in the open scientific literature, report that this substance is known to be found in human blood serum of employees exposed to the substance[1].

GK003126

[1] See (a) Gilliland FD, Mandel JS. Mortality Among Employees of a Perfluorooctanoic Acid Production Plant. J. Occup. Med. 1993;35:950 954; (b) Gilliland FD, Mandel JS. Serum Perfluorooctanoic Acid and

DUPONT HASKELL LAB 302 451 4828 04/03/01 09:05 02/03 NO:255

Per EPA's June 1991 TSCA Section 8(e) Reporting Guide, it is provided that "In deciding whether information is 'substantial risk' information, one must consider 1) the seriousness of the adverse effect, and 2) the fact or probability of the effect's occurrence." As stated above, no adverse health effects associated with this substance have been found among exposed employees. In the absence of an adverse effect, the information was deemed to not meet EPA TSCA Section 8(e) reporting criteria.

In addition, per the 1991 TSCA Section 8(e) Reporting Guide, information need not be submitted if it is published in the open scientific literature. As referenced above, prior published studies have reported that this substance is present in the blood serum of workers exposed to the substance. Also, in 1980, it was reported in the American Industrial Hygiene Association Journal that perfluorooctanoate anion was found in the blood of workers exposed to ammonium perfluorooctanoate[2]. The blood serum information provided in the DuPont submissions is not indicative of information not known to the Agency. It is consistent with information already published in the open scientific literature and as such, was deemed to not meet the criteria for TSCA section 8(e) reporting.

Please contact me directly if you need further clarification or wish to discuss this matter in more detail.

Very truly yours,

Gerald Kennedy

Hepatic Enzymes, Lipoproteins, and Cholesterol: A Study of Occupationally Exposed Men. Am. J. Ind. Med. 1996;29:560-568; (c) Olsen GW, Gilliland FD, Burlew MM, Burris JM, Mandel JS, Mandel JH. An Epidemiologic Investigation of Reproductive Hormones in Men with Occupational Exposure to Perfluorooctanoic Acid. J. Occup. Env. Med. 1998;40:614-622.

[2] Ubel FA, Sorenson SD, Roach, DE. Health Status of Plant Workers Exposed to Fluorochemicals – a Preliminary Report. American Industrial Hygiene Association Journal. 1980;41:584-589.

GK003127

04/03/01 09:05 [03/03 NO:255 302 451 4828 DUPONT HASKELL LAB

EXHIBIT C-54

Allen Weidman <AWeidman@socplas.org> on 02/26/2003 11:31:36 AM

To: David M Rurak/AE/DuPont@DuPont, "Frank Tortorici (E-mail)" <frank.tortorici@atofina.com>, "James Sullivan (E-mail)" <jvsullivan@mmm.com>, "Laird McBeth (E-mail)" <laird.mcbeth@solvay.com>, Miguel A Cardona/AE/DuPont@DuPont, "Noel Misa (E-mail)" <Noel_Misa@agfusa.com>, "Takayuki Nakamura (E-mail)" <tnakamura@daikin-america.com>

cc: "George Millet (E-mail)" <ghmillet@mmm.com>, "Harris, Lynne" <lharris@socplas.org>, L William Buxton/DuPont@DuPont, "Larry Wempe (E-mail)" <larry.wempe@atofina.com>, "Virginia Hubert (E-mail)" <virginia.hubert@solvay.com>, "Buffington, Jennifer" <jbuffing@socplas.org>, "Duncan, Don" <dduncan@socplas.org>, "Healey, Maureen" <MHealey@socplas.org>, "Limbach, Bonnie" <blimbach@socplas.org>, "Martinko, Marie" <Mmartink@socplas.org>

Subject: FW: FMG Communications materials.

Message to: Members, CWG
Allen Weidman, for distribution to FMG (Allen, please include this e-mail in forwarding the attachments.)

CWG Chair Diane Shomper has asked me to forward the attached materials to you for your consideration and action. It will help if you first read her cover memo, which explains the specific materials in the communications package. Please note that a number of these materials are the latest versions of "living documents" that have changed over the months as the issue and our activities have evolved. Most are reactive ("just in case") documents; some are for guidance in framing messages (i.e. the issues matrix) and some are proactive (i.e. communications to full FPD and to customers via letters from Don Duncan) and will require an FMG decision on whether and when to forward to the audiences noted. For your information, I also have attached a copy of the latest roster for the CWG, the keepers and distributors of communications-related knowledge within their individual companies.

I encourage everyone (if they haven't already) to create a special electronic file folder in which all the latest information/versions will be housed. This will help eliminate confusion going forward.

Everyone by now has seen the Columbus Dispatch reports, the flames of which are being flamed by the Environmental Working Group. The materials attached thus also will be helpful to individual companies in responding to those "don't you guys use this stuff?" questions. Questions specific to those stories, of course, are to be referred to Diane at DuPont. General questions about the issue and the FMG's activities can be referred to me, and I'll assemble the appropriate persons for response. Company-specific information will, of course, be addressed by the individual companies' designated spokesperson. At present, they are: Daikin: Al Damico as gatekeeper for Larry Galvin; Solvay: Ginny Hubert; Asahi: Noel Misa; Atofina: Jim Bell as gatekeeper for Frank Tortorici; DuPont: Diane Shomper as gatekeeper for Rich Angiullo; Dyneon and 3M: Rick Renner; W. L. Gore: Ed Schneider. Various companies indicated plans to designate/train additional spokespersons, and media training is in progress. The Issues Matrix also denotes technical sources of expertise available.

Just FYI: Ken Cook of the Environmental Working Group spoke yesterday at an NIH meeting in a session on "Media and Communications." In that session, he addressed how the EWG decides to communicate on an issue. He said it was when a regulatory situation may be opening up. As an example, he referred to PFOA, referencing what apparently is the Auer letter, which Cook described as "buried" in the Administrative record. He characterized the situation as being considered for accelerated review by the EPA. In explaining what PFOA is, he called it "a component of Teflon." He also talked about PFOS, an "obvious case" of when a group such as his gets involved.

<http://www.gifworld.com/searchsite/download?oid=1857741&fmt=JPG&kw=patriotism&at=190&ra=304&pd=PD-0033537&title=Gif World&rank=gifworld>

Bonnie Merrill Limbach
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To: FMG Communications Work Group and FMG

From: Diane R. Shomper, chairperson, CWG

Date: February 26, 2003

Re: Communications materials for review and action

As you know, EPA may announce its decision on whether it intends to proceed with a 4 (F) regulatory review of PFOA at any time, and press reports related to specific company issues have increased. It obviously is important that we remain as prepared as possible for inquiries we may receive from customers, employees, the media, local regulators and other audiences.

The following materials have been prepared to assist FMG members and their customers in responding to such inquiries. They are being provided to you for your final review and approval before being more widely distributed to the industry. (Please note that a number of these materials are "living documents" that have continued to evolve with the issue.) For this package, any substantive comments should be addressed to Bonnie Limbach at SPI by COB Monday, March 3.

The complete set of materials and their intended use is as follows:

1. **Message points.** These are for guidance in responding to media or other queries if EPA announces that it intends to proceed with a 4 (F) review.

2. **Standby statements.** There are two. One is for guidance and use in responding to media or other queries relating to industry's submission of a Letter of Intent to EPA. The other is for queries not related to the LoI.

3. **Issues Matrix.** This is for internal company use only. It is a summary of the principal issues relating to PFOA and industry's position on them. It can be used as a guide in responding to queries.

4. **Technical summary.** This is a summary and analysis of the existing health data relating to PFOA by the non-profit Environmental Health Research Foundation. It is a reassuring third-party assessment that can be used as a guide in responding to queries and also can be provided to customers, downstream companies and other interested parties.

5. **Duncan letters.** There are two letters from SPI President Don Duncan. One is a general "comfort" letter to reassure companies that the problem with EPA is being addressed. The second is a cover letter to the technical summary. ****Action required: FMG to decide if, when and how they want the letters and attachment to be distributed.**

6. **Uses and benefits of PFOA.** This is a summary of the societal benefits of PFOA and fluoropolymers for use in responding to media or others. It can also be distributed to all interested parties.

A final item, an employee-oriented overview of the health issues for the benefit of company health and human resources personnel, is in preparation and will be distributed after review by legal counsel.

Best regards.

[SPI Letterhead]

[date]

To: Members of the SPI Fluoropolymers Division

You are probably aware by now of interest that has been expressed by the U.S. Environmental Protection Agency regarding perfluorooctanoic acid (PFOA), an essential fluoropolymer processing aid. The purpose of this letter is to provide you with a brief update on the issue and the actions that SPI and its Fluoropolymers Manufacturers Group (FMG) have taken.

EPA's interest arose with the recent discovery by industry scientists, which was promptly reported to EPA, that trace levels of PFOA -- about 5 parts per billion -- had been found in random blood samples of the U.S. population.

Careful monitoring and research over a period of more than 20 years, including many studies published in the open scientific literature, have not revealed any adverse health effects on workers exposed to PFOA. Nor have any adverse health effects ever been reported among members of the public, whose exposures to PFOA are significantly less than those of workers.

Industry toxicologists and business leaders have been meeting with EPA over the past year and a half to examine all existing research on potential health effects related to PFOA, determine what additional research may be needed and improve overall stewardship of this compound. We hope to have formalized agreements on these issues in the near future.

Meanwhile, the industry has taken a number of important steps to reduce exposures. Fluoropolymer manufacturers have committed to reducing PFOA emissions worldwide by at least 50 percent by 2006. In addition, technology enhancements have enabled a 99-percent reduction in PFOA emissions from PFOA manufacturing in the United States.

We will continue to work closely with EPA on this issue and will report to you promptly on any regulatory or other significant developments. Please feel free to share this letter or the information contained herein with others in your organization or with your customers.

Sincerely,

Donald K. Duncan
President

2/26/03

STANDBY STATEMENT ON LOI
FOR USE IN RESPONDING TO POSSIBLE MEDIA QUERIES
PREVIOUS TO ANY FINAL OFFICIAL STATEMENT ON THE LOI.

Several American, European and Japanese makers of fluoropolymers and of the surfactants used in their production have, through The Society of the Plastics Industry, submitted documents to EPA outlining their commitment to further research on possible human exposures to perfluorooctanoic acid (PFOA) and its salts.

In recent years, industry had detected trace amounts of PFOA in blood samples in various parts of the United States and reported these findings to the EPA. The new research will focus on additional sampling and possible routes of exposure.

Extensive research and health monitoring of industry workers over many years has shown no evidence of adverse health effects from PFOA at worker exposure levels, and there are no indications of health effects among the general public, whose potential exposure would be considerably less.

Further, new technology used in manufacturing PFOA in the United States has almost totally eliminated emissions to the environment from this source. American and foreign companies that use PFOA to make fluoropolymers also have taken steps to substantially reduce emissions.

At this stage, the industry documents submitted to EPA are still in draft form and we are waiting for EPA and other inputs before finalizing them. We do not believe it would be helpful, or appropriate, to release them until they are in final form.

We can say, however, that the documents reflect the strong commitment of the industry to work in partnership with EPA to develop additional data on possible health or environmental effects of PFOA and its salts, including potential routes of exposure, as well as to take whatever additional measures may be advisable to reduce or prevent unnecessary exposure.

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EID825922

DRAFT ONLY (For discussion and revisions as necessary) **NOT FOR DISTRIBUTION**

Perfluorooctanoic acid (PFOA) Issues Matrix – **February 20, 2003**

Issue	FMG Position	Proof Points	**Industry/Third Party Spokespersons/** Company Experts	Comments
Industry Responsiveness	Industry is spending $ millions to reduce exposures even though no adverse human health or environmental effect has been discovered	Fluoropolymer manufacturers have committed to reducing PFOA emissions worldwide by at least 50 percent by 2006. In addition, technology enhancements have enabled a 99-percent reduction in PFOA emissions from PFOA manufacturing in the U.S.; industry has been conducting research for over 20 years and providing it to EPA and other regulatory agencies.	**B. Limbach/L. Harris (SPI)** and company contacts	Letters, presentations to EPA

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Product Uses	PFOA is used to make fluoropolymer (FP) plastics and synthetic rubber materials for applications in the aerospace, automotive, defense, telecommunications and other industries	Fluoropolymers made with PFOA are sold either as a liquid dispersion (for coating metal or glass cloth) or as a dry resin for making plastics. FP materials can withstand the temperatures inside baking ovens and in the engine compartments of jet aircraft. Their unique combination of heat, chemical and electrical resistance provides highly desirable properties needed in the event of fire, fluid release, electrical overload and similar emergencies.	**B. Limbach/L. Harris (SPI)** and company contacts	Letters, presentations to EPA, Guide to the Safe Handling of Fluoropolymer Resins, 1998, Guide to the Safe Handling of Fluoropolymer Dispersions, 2001
Alternative Chemistry	For most uses of PFOA, there is no viable alternative chemistry	FMG companies have conducted extensive research for alternatives over many years; none has been identified	**B. Limbach/L. Harris** and company contacts	Letters, presentations to EPA

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Ongoing Research	Although there is no evidence that current levels of exposure to PFOA cause adverse effects to human health or the environment, FMG members are committed to working in partnership with EPA on a proactive program to develop additional data	The proposed research program focuses on additional sampling and analysis of human blood; studies on protein binding, adsorption, desorption, elimination, mass balance and modeling in rats and humans; and long-term toxicity studies in fish and aquatic invertebrates	**B. Limbach/L. Harris Dr. Heinze (EHRF)**	Information provided to EPA
Letter of Intent (LoI)	Although there is no evidence that current levels of exposure to PFOA cause adverse effects to human health or the environment, FMG members are committed to working in partnership with EPA on a proactive research and product stewardship program outlined in a LoI	The LoI includes the research proposed above as well as a review of routes of exposure and product stewardship practices at sites where PFOA is manufactured, where it is used to make fluoropolymers, where fluoropolymer dispersions are used, and in fluoropolymer products made with PFOA	**B. Limbach/L. Harris Dr. Heinze (EHRF)**	LoI

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Blood	No evidence of adverse human health effects at ppb levels discovered	Levels in blood are 100s of times below those showing no adverse health effects in workers (workers' blood, mean = 2-3 ppm PFOA, general pop., mean = 0.005 ppm, 5 ppb)	**Dr. Heinze (EHRF)** **B. Limbach/L. Harris** Occupational Med. Sci. (TBD) Dr. Olsen/Dr. Butenhoff (3M) Dr. Murphy (Atofina) G. Kennedy (DuPont)	Olsen et al., 1998, 2000; data submitted to EPA
Worker Exposure	No evidence of adverse health effects at current level of exposure	Medical monitoring & studies of workers	**Dr. Heinze** Occupational Med. Sci. (TBD) Dr. Olsen/Dr. Butenhoff Dr. Murphy G. Kennedy	Olsen et al., 1998, 2000; data submitted to EPA

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Reproductive Health	There are no known adverse health effects from current levels of exposure	No adverse health effects in worker studies; recently completed two-generation study in rats shows PFOA does not affect reproductive processes	**Dr. Heinze** **Dr. Hood (U. of Alabama)** Dr. Butenhoff Dr. Murphy G. Kennedy	Two-generation study, other data submitted to EPA
Developmental Health	No evidence of adverse health effects at current level of exposure	No adverse health effects in worker studies; only effects seen in two-generation rat study were at dose levels many times higher than levels found in blood.	**Dr. Heinze** **Dr. Hood** Dr. Butenhoff Dr. Murphy G. Kennedy	Data submitted to EPA
Children's Health	There is no known adverse effect on children's health from current levels of exposure	Levels in blood are 100s of times below those showing no effects in workers (workers' blood, mean = 2-3 ppm PFOA, children, mean = 0.005 ppm, 5 ppb)	**Dr. Heinze** **Dr. Witorsch (U. VA Med. Col.)**	Olsen et al., 1998, 2000; data submitted to EPA

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Cancer	There is no evidence that PFOA causes cancer in humans	Studies of workers have not shown any increased cancer risk; PFOA is not genotoxic; liver tumors in rats produced by peroxisome proliferation unlikely to be relevant to humans, as are testicular and pancreatic tumors; mammary gland tumor incidence not increased over historical controls.	**Dr. Heinze** **TBD** Dr. Butenhoff Dr. Murphy G. Kennedy	Data submitted to EPA
Endocrine Disruption	There is no evidence that PFOA causes endocrine disruption	Hormonal effects not observed in two generation study	**Dr. Heinze** **Dr. Hood** Dr. Butenhoff Dr. Murphy G. Kennedy	Data submitted to EPA
HPV Program	PFOA is not an HPV material		**B. Limbach/L. Harris** and company contacts	Data submitted to EPA

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Persistence & Bioaccumulation	Based on existing data, PFOA does not meet EPA criteria for a persistent, bioaccumulative and toxic chemical	Two fish studies indicate PFOA shows little or no bioaccumulation potential (bioconcentration factors <10); levels in wildlife, where detected, are orders of magnitude below NOAELs (rats); levels in the river water, where detected, are orders of magnitude below acute toxicity levels; chronic eco-toxicity studies in progress	**Dr. Heinze**	PBT criteria in Fed. Reg.; data submitted to EPA

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Regulation	FMG member companies have submitted all relevant data to EPA; no current regulations specific to PFOA; is under review by EPA for possible regulation under TSCA; industry position is no specific regulation needed; industry committed to continuing work with EPA, other authorities internationally	Letters, presentations in EPA files	**B. Limbach/L. Harris** and company contacts	Data submitted to EPA
Possible TSCA 4(f) regulation	There is no evidence that current levels of exposure to PFOA cause adverse effects to human health or the environment and thus little justification for specific regulation	FMG members have submitted all relevant data to EPA and are committed to working in partnership with EPA on a proactive research and product stewardship program outlined in a Letter of Intent (LoI)	**B. Limbach/L. Harris** and company contacts	Data submitted to EPA, LoI

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Routes of Exposure	FMG members have proposed to EPA a review of routes of exposure and product stewardship practices at sites where PFOA is manufactured and used, as well as in fluoro-polymer products made with PFOA	The Letter of Intent (LoI) includes a review of routes of exposure and product stewardship practices at sites where PFOA is manufactured, where it is used to make fluoropolymers, where fluoropolymer dispersions are used, and in fluoropolymer products made with PFOA	**B. Limbach/L. Harris** and company contacts	Data submitted to EPA, LoI
Relationship to Telomers	The Telomer parties have committed to EPA to research the possibility of the transformation of fluorochemical products known as "telomers" into PFOA	Further information may be obtained by contacting the Telomers parties contact	**The Telomer parties' contact (need to identify)**	Umbrella LoI
Relationship to other FP Processing Aids	A small amount of FPAs are made with a different compound, called APFN	Further information may be obtained by contacting the APFN contact	**Need to identify contact (L. Harris?)**	

Communication	FMG member companies are proactively informing employees, regulators and customers on this issue	Employee meetings; submission of data and meetings with EPA. Letters to customers	**B. Limbach/L. Harris** and company contacts	

2/26/03

FMG STANDBY STATEMENT FOR GENERAL MEDIA QUERIES

Members of The Society of the Plastics Industry's Fluoropolymers Manufacturers Group have been working cooperatively with the U.S. Environmental Protection Agency over the past year and a half.

The subject of these meetings has been the recent discovery by industry scientists of trace levels of a chemical known as perfluorooctanoic acid (PFOA) in random blood samples of the US population. The levels of PFOA were extremely low, approximately 5 parts per billion. This is equivalent to a single teaspoon of water in an Olympic-size swimming pool (50 meters long, 40 feet wide, 5 feet deep, holding 260 thousand gallons of water).

PFOA is used primarily in industrial processes. For the past 50 years it has been used in the polymerization process for making fluoropolymer plastics and synthetic rubber materials for applications in the aerospace, defense, automotive, telecommunications and other industries.

Careful monitoring and research over a period of more than 20 years, including many studies published in the open scientific literature, have revealed no adverse effects on the health of workers exposed to PFOA. Nor have any adverse health effects ever been reported among members of the public, whose exposures to PFOA are significantly less than those of workers.

Industry scientists have been meeting with EPA to examine all existing research on potential health effects and determine what additional research may be needed.

Meanwhile, the industry has taken a number of important steps to reduce exposures. Fluoropolymer manufacturers have committed to reducing PFOA emissions worldwide by at least 50 percent by 2006. In addition, technology enhancements have enabled a 99-percent reduction in PFOA emissions from PFOA manufacturing in the United States.

# # #

2/26/03

MESSAGE POINTS IF 4F IS ANNOUNCED

- **We have known for some time that this was under consideration.**
- **It is a step in a process that began some 18 months ago.**
- **Industry has been working closely with EPA and will continue to do so.**
- **Our relations are cordial and cooperative.**
- **We have the same goals: to protect public health and the environment.**
- **The announcement of the 4F process marks the official beginning of a 180-day review period to determine whether further research is needed, or whether any regulations are needed.**
- **Industry has provided and continues to provide EPA with extensive research, including the results of blood sampling in workers and among the general public.**
- **This research indicates no adverse health effects at the extremely low levels of PFOA found among exposed workers or among members of the public.**
- **Industry already has taken steps to reduce the amount of PFOA that escapes into the environment.**
- **Emissions from PFOA production in the United States have been almost totally eliminated (99%) through the use of new technology.**
- **Fluoropolymer manufacturers around the world have committed to major reductions in PFOA emissions from their facilities.**
- **There is no viable alternative to PFOA for the manufacturing of fluoropolymers needed in many high-tech, high performance technologies in critical applications such as aerospace, computers, defense, energy and telecommunications.**

ENVIRONMENTAL HEALTH RESEARCH FOUNDATION

A NONPROFIT RESEARCH FOUNDATION SPECIALIZING IN HEALTH AND ENVIRONMENTAL SCIENCE

JOHN E HEINZE PhD
Executive Director

February 20, 2003

Mr. Donald K. Duncan, President
The Society of the Plastics Industry, Inc.
Suite 600K
1801 K Street, NW
Washington, DC 20006-1301

Dear Mr. Duncan:

Please accept this report prepared by the Environmental Health Research Foundation regarding human health data on perfluorooctanoic acid (PFOA).

The report provides a summary and analysis of the considerable scientific information available on this material.

Please feel free to distribute this report within SPI, and to member companies and others as you determine may be appropriate.

We of course would be happy to answer any questions on the report. You may contact me directly at either of our phone numbers (703-331-5500 or 202-737-0337).

Sincerely,

John E. Heinze, Ph.D.

Enclosure: Summary and Analysis of Health Data on Perfluorooctanoic Acid (PFOA)

7525 PRESIDENTIAL LANE, MANASSAS, VA 20109 PHONE: 703-331-5500/202-737-0337

ENVIRONMENTAL HEALTH RESEARCH FOUNDATION

A NONPROFIT RESEARCH FOUNDATION SPECIALIZING IN HEALTH AND ENVIRONMENTAL SCIENCE

Summary and Analysis of Health Data on Perfluorooctanoic Acid (PFOA)

February 20, 2003

John E. Heinze, Ph.D.
Executive Director

7525 PRESIDENTIAL LANE, MANASSAS, VA 20109 PHONE: 703-331-5500/202-737-0337

Summary and Analysis of Health Data on Perfluorooctanoic Acid (PFOA)

Summary

The Environmental Health Research Foundation has reviewed the extensive database of studies on perfluorooctanoic acid (PFOA) and assessed their significance for human health. These studies include both laboratory animal studies and studies of employees at fluorochemical production plants that manufacture or use PFOA. The laboratory animal studies include multiple reproductive and developmental studies, including a two-generation study, and two 2-year feeding (chronic oral toxicity/tumorigenicity) studies. The worker studies include evaluations of health insurance claims (episodes-of-care data), medical surveillance (standard health assessments as well as evaluations of biochemical parameters affected in laboratory animal studies) and mortality (observed versus expected causes of death). The results of these studies showed no evidence of adverse health effects to workers or the general population at current PFOA exposure levels. This finding is remarkable given the large number of state-of-the-art studies conducted to test for possible health effects.

Detailed Analysis

Introduction

The Fluoropolymers Manufacturers Group of The Society of the Plastics Industry, Inc., requested the Environmental Health Research Foundation (EHRF) to conduct an independent review of the available science on possible health effects of exposure to perfluorooctanoic acid (PFOA). This compound provides unique and critical performance properties for fluoropolymer plastics and synthetic rubber materials for essential applications in the automotive, defense, telecommunications and other industries.

EHRF is a non-profit scientific consulting organization specializing in the analysis and review of health effects of environmental exposures. EHRF personnel have more than 25-years' experience in evaluating health and environmental effects for the plastics, consumer products and ingredient supplier industries. The review is based on:

1) the document prepared by the Association of Plastics Manufacturers of Europe and the Society of the Plastics Industry (Butenhoff et al., 2002) provided to the US Environmental Protection Agency (EPA) as well as the reports and studies cited in this document,
2) the November 4, 2002 EPA Draft Hazard Assessment (EPA, 2002), and
3) the over 1100 reports provided to EPA as part of Administrative Record 226.

PFOA has been detected at trace levels (5 parts per billion) in serum of blood donors in the U.S. (Olsen et al., 2002). An assessment of the health risk posed by this exposure has been conducted based on a review of studies of the health of workers at fluorochemical manufacturing plants, who have been found to have PFOA levels in their blood hundreds of times higher than levels in the U.S. population, and laboratory animal studies.

Reproductive and Developmental Studies

Employee Studies

A 1993-1998 study of workers at a fluorochemical plant at which PFOA had been used for many years found no increase in episodes-of-care[1] for pregnancy and its potential complications, for congenital anomalies, or for perinatal disorders among female employees (122 total) compared to those at a non-fluorochemical plant (101 total) at the same site (Olsen et al., 2001b). There is no evidence from this study to suggest increases in reproductive and developmental effects associated with exposure to PFOA.

Laboratory Animal Studies

A two-generation reproduction and developmental study (York, 2002) was recently conducted in laboratory rats exposed to oral PFOA doses of 1, 3, 10 or 30 milligrams per kilogram body weight per day (mg/kg bw d). In the parental animals, reduced weight gains and liver, kidney and spleen weight changes were observed in the males at all doses. Females showed reduced weight gains only at the highest dose. At the highest dose level (30 mg/kg bw d), a number of effects were observed on the offspring including decreased body weights, increased mortality (only in first offspring generation) and delayed sexual development in females (vaginal opening) and males (preputial separation). There were no effects observed on the offspring at lower doses and no effects on mating or fertility at any dose in either generation.

The increased incidence of offspring mortality at 30 mg/kg bw d was most likely a result of a compromised nutritional status as reflected by reduced maternal body weights and reduced body weights of the offspring. This phenomenon has been observed in certain pharmaceutical drugs and other substances that have a similar mechanism of action (peroxisome proliferation[2]).

Similarly, delays in sexual development at 30 mg/kg bw d were most likely the result of delayed growth of the offspring since decreased body weights were noted throughout the lactation period. Decreased weights can result in non-specific delays in sexual maturity in rats (Butenhoff et al., 2002 and references cited therein).

Three developmental toxicity studies (Gortner, 1981; Gortner, 1982; Staples et al., 1984) conducted in rats or rabbits by oral or inhalation exposure showed no evidence that

[1] An episode of care is defined as a series of medical events related to a particular health problem that exists continuously for a period of time. For instance, an episode of care would include an initial doctor's office visit, emergency room treatment or hospital admission as well as follow-up doctor's visits, etc. related to the same medical condition.

[2] Treatment of rodents with peroxisome proliferators initiates a characteristic sequence of morphological and biochemical events in the liver and to a lesser extent the kidneys. Morphological changes include swelling of liver cells due to increases in the number and size of subcellular components, especially peroxisomes (organelles involved in production and decomposition of the oxidant hydrogen peroxide). Biochemical changes include changes in fat (lipid) metabolism. Higher doses lead to liver damage. Rats and mice are highly, perhaps uniquely responsive to the effects of peroxisome proliferators; primates and humans seem to be practically non responsive.

maternal exposure to PFOA is uniquely hazardous to the developing fetus or offspring. Maternal body weight reductions and increased mortality were observed at the highest PFOA doses tested (as expected), but no effects were observed on the maternal animals, fetuses or offspring at lower PFOA doses.

The overall no observed adverse effect level (NOAEL) for PFOA for reproduction and development from these studies, 10 mg/kg bw d, produces PFOA levels in the blood of the rats many times higher than blood levels of the U.S. population (Olsen et al., 2002). Consequently, laboratory animal studies provide no evidence of adverse reproductive or developmental health effects at current human exposure levels.

Tumorigenicity Studies

Prostate

An epidemiological investigation of a perfluorochemical production workforce associated prostate cancer mortality with duration of employment (Gilliland, 1992, Gilliland & Mandel, 1993). The US EPA characterized this association as weak (EPA, 2002). A major limitation of the study was that exposure to PFOA was not quantified and only one of the studied employees had actually worked directly in the PFOA production building (Olsen et al., 1998). In a follow-up study among employees with definite or possible exposure to PFOA (Alexander, 2001a), no association was found between prostate cancer mortality and duration of employment, suggesting that the weak association observed in the earlier study was not due to PFOA exposure.

At a second perfluorochemical production plant, an episodes-of-care investigation suggested an elevated risk for prostate malignant neoplasms, but this was contradicted by the finding of no increased risk of prostate enlargement (hypertrophy), a common pre-cancer condition (Olsen et al., 2001b). Levels of estradiol and other hormones that might be linked to prostate disease were also unchanged (Olsen et al., 1998). The incidence of prostrate cancer mortality (one case expected by chance, none observed) was not elevated (Alexander, 2001b).

Prostate cancer was not observed in either of the two-year feeding studies conducted in rats (Riker, 1983; Biegel et al., 2001).

In summary, neither the worker studies nor the laboratory animal studies provide evidence that exposure to PFOA increases the risk of prostate cancer.

Liver

In a two-year feeding study conducted in rats, PFOA increased the incidence of benign tumors (adenoma) of liver cells (Biegel et al., 2001). However, these are unlikely to be caused by direct interaction of PFOA with cellular DNA as PFOA is not genotoxic (Butenhoff et al., 2002 and references cited therein). Peroxisome proliferation (increased peroxisome activity and liver weights) was observed in this study and is likely the cause

of the liver tumors. Because humans are much less sensitive to peroxisome proliferation than rats, it is unlikely that the liver tumors in rats are relevant to humans.

This conclusion has been confirmed by several worker studies that have investigated the possible association between liver cancer or liver disease and PFOA exposure and have shown no association. These include:

1) Studies of liver cancer at two fluorochemical production facilities (Alexander, 2001a,b) showing no increase in risk,
2) An episode-of-care study showing no difference in reported disorders of the liver (cirrhosis and hepatitis) between plant fluorochemical workforce and non-exposed employees at the same manufacturing site (Olsen et al. 2001b) and
3) Studies of liver function among employees at two fluorochemical plants showing no changes in liver enzymes or bilirubin associated with PFOA levels in blood (Gilliland & Mandel, 1996; Olsen et al., 2000, 2003).

The worker studies provide no evidence that exposure to PFOA increases the risk of liver disease or cancer.

Testes

In both two-year feeding studies in rats, PFOA increased cell growth (hyperplasia) and benign tumors (adenomas) of testicular Leydig cells (Riker, 1983; Biegel et al., 2001). The mechanism for this type of tumor formation may be a sustained increase in estradiol levels in the testes, an effect that has been observed with other peroxisome proliferators (Butenhoff et al., 2002 and references cited therein).

Leydig cell tumors are rare in humans (about 2.5% of all testicular cancers, Schottenfeld, 1996). The relevance of these tumors and of the mechanism of action to humans has not been demonstrated.

An episode-of-care study among employees at a fluorochemical manufacturing facility from 1993-1998 found two individuals with health claims coded to testicular cancer versus 0.6 expected (Olsen et al., 1998). However, no deaths due to testicular cancer were observed among employees (0.2 expected) at this plant over a 38-year study period (Olsen et al., 2001b). At a second fluorochemical production site, only one death due to testicular cancer was observed (versus 0.4 expected) among employees during a 50-year study period (Alexander et al., 2001a). A study of hormone levels in employees at this plant found no changes in estradiol or other hormones that might be associated with testicular cancer (Olsen et al., 1998).

In conclusion, there is no evidence of a relationship between PFOA exposure and increased testicular cancer risk in humans.

Pancreas

In a two-year feeding study in rats (Biegel et al., 2001), PFOA increased the incidence of pancreatic acinar cell adenomas and combined adenomas/carcinomas (malignant tumors). Other peroxisome proliferators have been observed to induce this type of pancreatic

tumor in rodents suggesting that the mechanism of induction is related to peroxisome proliferation (Butenhoff et al., 2002 and references cited therein). The mechanism may involve changes in levels of steroid hormones such as cortisol and/or growth factors such as cholecystokinin (CCK).

This type of tumor is rare in humans and the relevance of the tumors observed in rats to human cancer has not been demonstrated.

Among 1500 employees with probable PFOA exposure at a fluorochemical production facility, there were six deaths attributed to pancreatic cancer compared to 4.8 expected (Alexander et al. 2001a). Among 182 employees with definite exposure to PFOA at this same facility, there was one death reported for pancreatic cancer compared to 0.8 expected. There were no changes in cortisol steroid hormone levels or CCK that correlated with PFOA levels in blood samples from these employees (Olsen et al., 1998, 2000).

At a second fluorochemical production facility, there were no deaths attributable to pancreatic cancer among the 1065 employees (one expected, Alexander et al., 2001b).

In short, there is no evidence of a relationship between PFOA exposure and pancreatic cancer in humans.

Breast

In a two-year feeding study in rats, the incidence of mammary gland tumors appeared to be elevated compared to the controls (Riker, 1983). However, comparison to background levels in other studies indicated that the incidence of mammary gland tumors is rather variable and both the control and test incidence rates were within historic control levels (Butenhoff et al., 2002 and references cited therein). In a second two-year feeding study in rats, the incidence of mammary gland tumors was within background (control) levels and not elevated (Biegel et al., 2001).

Non-malignant disorders of the breast (mostly fibrocystic disease) were slightly higher among employees at a fluorochemical production facility as the episodes-of-care risk ratio was 1.6 among fluorochemical plant employees compared to non-fluorochemical employees at the same site (Olsen et al., 2001b). For benign neoplasms, the risk ratio was nearly identical, 1.1, comparing fluorochemical and non-fluorochemical employees. There were two episodes of care for breast cancer (versus 3.5 expected) among fluorochemical employees and zero episodes of care (versus 4 expected) among non-fluorochemical employees. There have been no breast cancer deaths (versus 0.9 expected) (Alexander, 2001b).

Among employees with probable PFOA exposure at a second fluorochemical production facility, there have been two breast cancer deaths versus 3.6 expected (Alexander et al., 2001a). Among those with definite PFOA exposure, there have been no breast cancer deaths versus 0.2 expected.

In conclusion, there is no evidence that exposure to PFOA increases the incidence of breast cancer in humans.

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[SPI Letterhead]

To: SPI FMG

From: Don Duncan

Date: February 28, 2003

Enclosed for your information is a recently completed overview and independent analysis by the Environmental Health Research Foundation (EHRF) of the extensive database of studies examining possible human health effects associated with exposure to perfluorooctanoic acid (PFOA), an essential fluoropolymer processing aid.

The EHRF analysis finds there is no evidence of adverse human health effects associated with current worker or general public exposures to PFOA.

The studies reviewed here form the basis of the industry's belief that there is no evidence of adverse human health effects from current levels of exposure to PFOA, and that the recent and planned major reductions in PFOA manufacturing emissions will further reduce the possibility of any such effect.

Although we are still working with the U.S. Environmental Protection Agency to determine needs for additional research (for example, to better characterize routes of exposure), you may find this third-party analysis of the current science a useful reference within your company and for responding to questions you may receive from customers or others, including the technical or trade media.

Sincerely,

Donald K. Duncan
President

2/25/03

STANDBY STATEMENT ON THE SOCIETAL BENEFITS OF PFOA AND FLUOROPOLYMERS

Perfluorooctanoic acid (PFOA) and its salts are processing aids essential to the manufacture of fluoropolymer plastics and synthetic rubber materials (elastomers) for the

- defense/aerospace
- automotive
- electronics/semiconductor
- telecommunications
- chemical/petrochemical
- power generation/pollution control and
- consumer product industries.

Fluoropolymers can withstand the temperatures inside baking ovens and in the engine compartments of jet aircraft. Fluoropolymers also have high resistance to a broad range of fuels, solvents and corrosive chemicals, and excellent electrical insulating properties. These unique properties provide critical performance characteristics needed to prevent fire, fluid release, electrical overload or similar emergencies in many high performance applications. This unique combination of properties yields an extremely versatile family of materials that allows applications that would not otherwise be possible.

The types of products made from fluoropolymers include:

- space apparel
- wire and cable insulation, including wiring for satellites
- seals and bushings in motors
- hydraulic, fuel and brake hoses and tubing
- control cables
- computer and telecommunications wiring including fiber optic and coax cables
- high-purity fluid handling equipment
- clean room garments
- tank linings and reactor vessels
- valves, piping and filters
- gaskets for pumps
- downhole drilling seals
- filter bags and expansion joints
- non-stick cookware and bakeware, and
- waterproof/breathable clothing.

No substitute for PFOA has been identified for making these fluoropolymers.

Message to: Members, CWG
Allen Weidman, for distribution to FMG (Allen, please include this e-mail in forwarding the attachments.)

CWG Chair Diane Shomper has asked me to forward the attached materials to you for your consideration and action. It will help if you first read her cover memo, which explains the specific materials in the communications package. Please note that a number of these materials are the latest versions of "living documents" that have changed over the months as the issue and our activities have evolved. Most are reactive ("just in case") documents; some are for guidance in framing messages (i.e. the issues matrix) and some are proactive (i.e. communications to full FPD and to customers via letters from Don Duncan) and will require an FMG decision on whether and when to forward to the audiences noted. For your information, I also have attached a copy of the latest roster for the CWG, the keepers and distributors of communications-related knowledge within their individual companies.

I encourage everyone (if they haven't already) to create a special electronic file folder in which all the latest information/versions will be housed. This will help eliminate confusion going forward.

Everyone by now has seen the *Columbus Dispatch* reports, the flames of which are being flamed by the Environmental Working Group. The materials attached thus also will be helpful to individual companies in responding to those "don't you guys use this stuff?" questions. Questions specific to those stories, of course, are to be referred to Diane at DuPont. General questions about the issue and the FMG's activities can be referred to me, and I'll assemble the appropriate persons for response. Company-specific information will, of course, be addressed by the individual companies' designated spokesperson. At present, they are: Daikin: Al Damico as gatekeeper for Larry Galvin; Solvay: Ginny Hubert; Asahi: Noel Misa; Atofina: Jim Bell as gatekeeper for Frank Tortorici; DuPont: Diane Shomper as gatekeeper for Rich Angiullo; Dyneon and 3M: Rick Renner; W. L. Gore: Ed Schneider. Various companies indicated plans to designate/train additional spokespersons, and media training is in progress. The Issues Matrix also denotes technical sources of expertise available.

Just FYI: Ken Cook of the Environmental Working Group spoke yesterday at an NIH meeting in a session on "Media and Communications." In that session, he addressed how the EWG decides to communicate on an issue. He said it was when a regulatory situation may be opening up. As an example, he referred to PFOA, referencing what apparently is the Auer letter, which Cook described as "buried" in the Administrative record. He characterized the situation as being considered for accelerated review by the EPA. In explaining what PFOA is, he called it "a component of Teflon." He also talked about PFOS, an "obvious case" of when a group such as his gets involved.

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EXHIBIT C-55

George A Ainsley
03/19/2003 06:39 AM

To: Paul J Bossert/AE/DuPont@DuPont, Robert W Rickard/AE/DuPont@DuPont, Diane R Shomper/AE/DuPont@DuPont, H David Ramsey/SE/DuPont@DuPont, Dawn D Jackson/CL/DuPont@DuPont, R Clifton Webb/AE/DuPont@DuPont, David M Rurak/AE/DuPont@DuPont, Robbin Banerjee/AE/DuPont@DuPont, Andrew S Hartten/AE/DuPont@DuPont, JGOLLEHON@CHARLESRYAN.COM, msheppard@charlesryan.com, jvieweg@charlesryan.com, Kelli H Kukura/AE/DuPont@DuPont
cc: sfennell@steptoe.com
Subject: Media Presentation

Attached is the final version of the presentation made to the local media at Washington Works March 18.

Charts for 3-18-03 Media Briefing.

DuPont Washington Works

Welcome

MEDIA UPDATE

March 18, 2003

DuPont Washington Works

Paul J. Bossert, Jr.

Washington Works Plant Manager

DuPont Washington Works

- Overview
- Website update
- Emissions reduction progress
- Assessing the Science
- Toxicology, Human Exposure

DuPont Washington Works

DuPont Core Values

- Commitment to safety, health and environmental stewardship.
- Value and respect for people.
- High degree of ethics in all business practices.

DuPont Washington Works

DuPont's Commitment

- Respond to the public openly, honestly, and accurately.
- Work with state, federal and global regulatory agencies to

  expand knowledge of C-8 and improve stewardship.
- Further reduce emissions at Washington Works.
- Continue to maintain an operation at Washington Works that

  is safe for employees, the public and the environment.

DuPont Washington Works

Announcing Updates to the C-8 Information Website (www.c-8inform.com)

- New letter from Plant Manager Paul Bossert
- Emissions Reduction Progress Report
- Current news releases
- Recent letters from regulatory agencies
- The latest employee communications

DuPont Washington Works

Emissions Reduction Progress

	1999	2002	Reduction
Air	31,209	14,480	53.6%
Water	55,597	5,688	89.8%
Total	86,806	20,168	76.8%

* *Measurements in pounds*

DuPont Washington Works

Assessing the Science

Dr. Robert W. Rickard

Director of DuPont Haskell Laboratory for Health and Environmental Sciences

DuPont Washington Works

What We Would Like to Clarify

- Laboratory and worker studies assessing health effects of
  C-8
- Workers and community exposure
- Safeguards to protect human health and the environment

DuPont Washington Works

Health Effects

We are confident that there are no health effects associated with C-8 exposure.

Basis for Confidence:

Hazard Assessment	+	Exposure Assessment	=	Risk

DuPont Washington Works

Analysis of C-8 Health Effects

	Yes	No
Biopersistent	X	
Bioaccumulative		X
Animal Carcinogen	X	
Human Carcinogen		X
Developmental Toxin		X
Reproductive Toxin		X
Genetic Toxin		X

DuPont Washington Works

Assessing the Science

Toxicology

- Extensive database > 200 reports referenced in EPA Hazard Assessment
- Animal studies are designed to cause an effect
  - Is the effect relevant to humans?
  - Is the dose relevant to human exposure?

DuPont Washington Works

Assessing the Science

Is the effect relevant?

- Most sensitive animal model - rat
- Most sensitive effect - liver enlargement
- Do we understand why C-8 causes liver enlargement in rats?
  - Yes - induces peroxisome proliferation
- Is this mechanism relevant to humans?
  - Unlikely

DuPont Washington Works

Society of Toxicology

March 2003

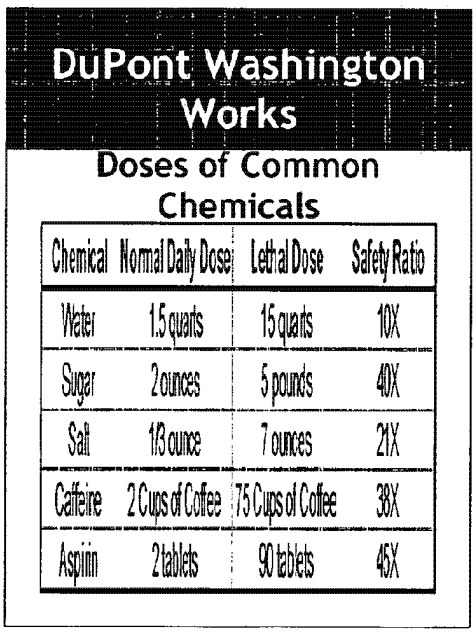
Conclusion: Humans appear to be non-responsive to the adverse effects of peroxisome proliferation

DuPont Washington Works

Assessing the Science

Is the dose (in animal studies) relev

Effect (Animal Model)	No Observed Effect Level	Equivalent Human Consumption Gal. of Water with 3 ppb C-8
Chronic-liver (rat and primate)	0.5 mg/kg	>2,000 gal. per day
Cancer (rat)	2.0 mg/kg	>8,000 gal. per day
Reproduction/ Developmental (rat)	10 mg/kg	>40,000 gal. per day



DuPont Washington Works

Doses of Common Chemicals

Chemical	Normal Daily Dose	Lethal Dose	Safety Ratio
Water	1.5 quarts	15 quarts	10X
Sugar	2 ounces	5 pounds	40X
Salt	1/3 ounce	7 ounces	21X
Caffeine	2 Cups of Coffee	75 Cups of Coffee	38X
Aspirin	2 tablets	90 tablets	45X

DuPont Washington Works

Assessing the Science

Human Data

- Extensive database
- Six published studies (1980-2001)
- New study due March 2003
- 3M workers with blood levels up to 100 ppm (maximum)

No health effects identified

DuPont Washington Works

Assessing the Science

Human Data

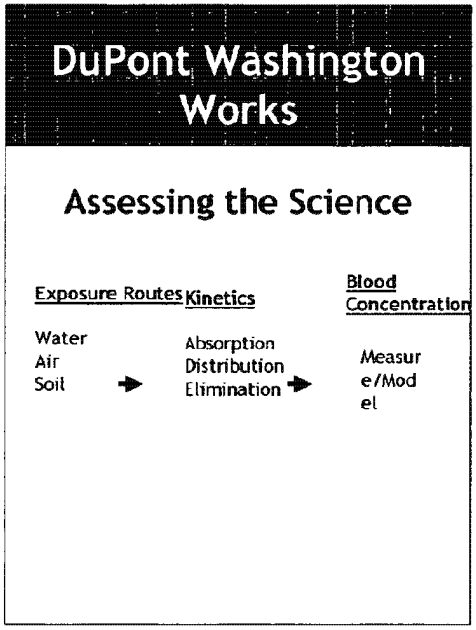
Endpoints Evaluated

Mortality	No effects
Cancer	No effects
Liver Enzymes	No effects
Cholesterol	No effects
Reproductive Hormones	No effects
Growth Hormones	No effects

DuPont Washington Works

Assessing the Science

- C-8 is biopersistent
- Half-life in humans is 4.4 years +/- 3.5 years
- Half-life in animals is days, weeks or months


DuPont Washington Works
Assessing the Science
Exposure Routes
Kinetics
Blood Concentration
Water
Air
Soil
Absorption
Distribution
Elimination
Measur
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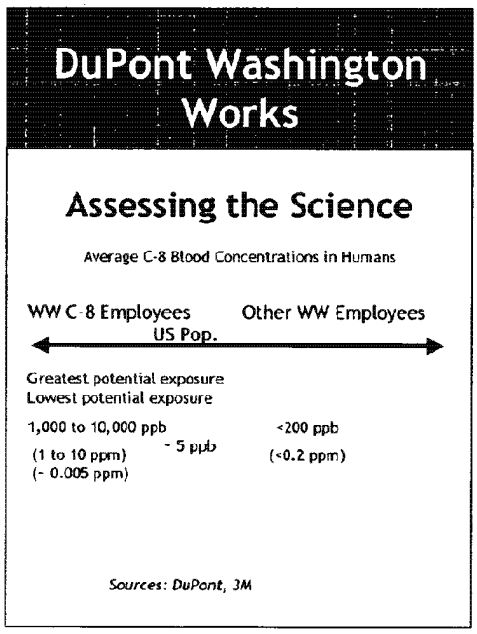
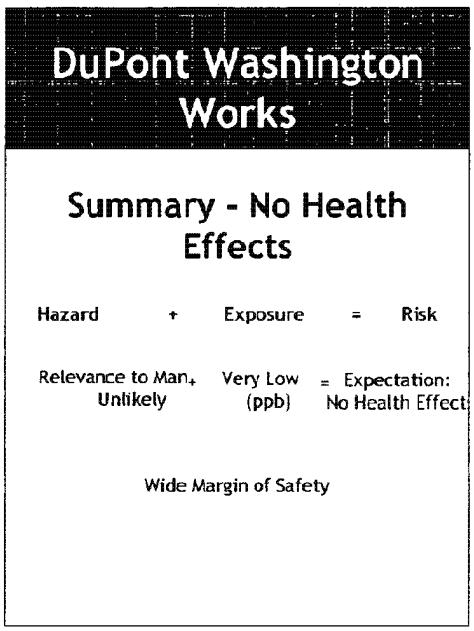

DuPont Washington Works
Assessing the Science
Average C-8 Blood Concentrations in Humans
WW C-8 Employees
US Pop.
Other WW Employees
Greatest potential exposure
Lowest potential exposure
1,000 to 10,000 ppb
(1 to 10 ppm)
(~ 0.005 ppm)
~ 5 ppb
<200 ppb
(<0.2 ppm)
Sources: DuPont, 3M

DuPont Washington Works

Assessing the Science

Next Steps

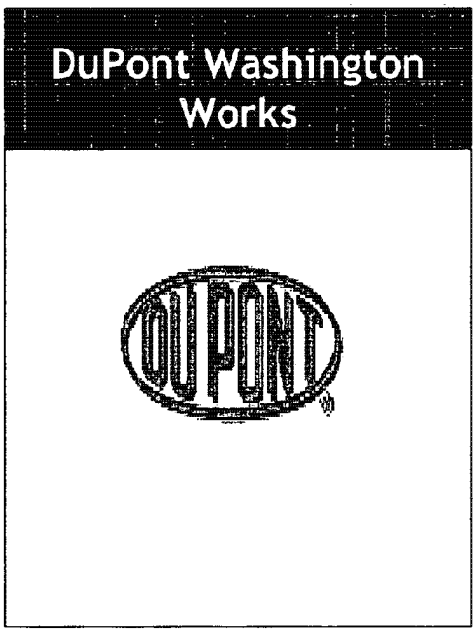
- Complete animal kinetic studies
- Complete blood binding studies
- Continue to evaluate all available data
- Build and validate model if feasible
- Share results with state, federal and global regulatory agencies (ongoing)


DuPont Washington Works
Summary - No Health Effects
Hazard + Exposure = Risk
Relevance to Man, Unlikely
Very Low (ppb)
= Expectation: No Health Effect
Wide Margin of Safety

DuPont Washington Works

Conclusions - Health Effects

- *In more than fifty years of C-8 use by DuPont and others, there are no known adverse health effects in workers associated with C-8*
- *Workers that manufacture and use C-8 have the highest potential for exposure*
- *Community exposure is significantly lower than in the workplace*
- *C-8 is not a human health issue*


DuPont Washington Works
DUPONT

DuPont Washington Works

Modeling vs. Data

The Claim: Ohio residents may have significant concentration of C-8 in their blood, according to a DuPont model.

The Facts: There are no data to support the claim. The model significantly overestimates potential concentration of C-8 in area residents' blood.

EXHIBIT C-56

Please print and share this information with any employee who may not have access to E-Mail or Lotus Notes

From: Wash Works Comm_Center on 03/19/2003 01:47 PM

Sent by: Rosalie S Wilson

To: Wash Works Comm_Center/Mail-in/DuPont@DuPont
cc:
Subject: Special Edition Message from the Plant Manager -- C-8 Information

TO ALL EMPLOYEES:

You may have noticed yesterday evening and this morning considerable news coverage of C-8 information that I have previously shared with you in my all-employee communications. We had an opportunity to provide that same information to the media yesterday when we held a media briefing here on-site. We reported on our progress to reduce C-8 emissions, and we corrected and clarified a number of recent reports that have included inaccuracies or information taken out of context. Because there has been such a focus on whether or not C-8 exposure causes health effects, Dr. Robert Rickard, Director of the DuPont Haskell Laboratory for Health and Environmental Sciences, participated in the briefing.

Dr. Rickard explained to the media -- as we have stated many times before -- that in 50 years of C-8 use by DuPont and others, there have been no observed adverse human health effects associated with C-8 exposure. He also explained that we *do* see health effects in laboratory animals because animal studies are *designed* to cause effects. The three effects most often mentioned are chronic liver effects, cancer, and reproductive or developmental effects. To put the animal testing in proper perspective, consider this: At a 3-part per billion concentration of C-8 in drinking water, in order for a human to be exposed to the amount of C-8 to which rats are exposed in a chronic-liver effect study, the human would have to drink more than 2,000 gallons of water a day. For the cancer study, it would be more than 8,000 gallons a day; and for the reproductive or developmental effect study, it would be more than 40,000 gallons a day. At such low concentrations, Dr. Rickard explained, the effects seen in test animals are not relevant to humans.

Along with other new information, the PowerPoint® presentation from which Dr. Rickard and I spoke yesterday will be available on our www.C8inform.com web site later today. I have attached the news release that we provided yesterday, and it will also be available on the web site. I encourage you to review the new material on the site, to be well informed, and to get in touch with me if you have questions.

Paul Bossert

3-18-03 Media Update News Release -- FINAL.

PARKERSBURG, W. Va., March 18, 2003 —— In a news briefing at DuPont Washington Works today, Plant Manager Paul Bossert and Director of DuPont Haskell Laboratory for Health & Environmental Sciences Robert Rickard, PhD., reported progress on DuPont's reduction of C8 emissions and described the scientific data that support DuPont's position that its use of C8 does not have an adverse effect on human health or the environment. C8 is an essential processing aid used by DuPont and others in the manufacture of fluoropolymers.

In particular, Bossert and Rickard wanted to correct several misconceptions about C8 that they have heard. "I frequently hear that because C8 is unregulated, little is known about the material or its health effects. That is not true. We know a great deal about C8, and we know that in more than 50 years of C8 use by DuPont and others, there have been no known adverse human health effects," Bossert said. Rickard later elaborated on the statement with references to the extensive database about C8, including 200 reports referenced in USEPA hazard assessment documents.

Bossert also disputed the statement that C8 causes cancer in animals, explaining that such statements taken out of context cause undue concern in the community. "The USEPA stated in March 2002 in its Consent Order with DuPont that studies have determined that 'C-8 in sufficient doses, i.e., considering both amount and duration of exposure, is toxic to animals through ingestion, inhalation, and dermal contact.' It is extremely important," noted Bossert, "that a statement about health effects includes a reference to dose and duration."

-more-

Rickard later explained that animal studies are *designed* to cause an effect and addressed whether the results of the studies are relevant to human health. "The data support that at the extremely low concentrations to which humans are exposed, the effects are not relevant to humans," Rickard said, citing various studies and reports.

Bossert also reported on DuPont's on-going efforts to reduce emissions of C8 to the environment. According to a recent progress report submitted to the West Virginia Division of Environmental Protection, DuPont achieved nearly a 77 percent total reduction in C8 emissions from 1999 to 2002. Emissions to water were cut by nearly 90 percent, while emissions to air were reduced more than 50 percent. The company expects to achieve a 90 percent reduction in total C8 emissions by the end of 2004.

"DuPont's commitment to reduce C8 emissions is demonstrated by our investment in abatement facilities and our efforts to develop the technology needed to reduce, recycle, and reuse this important material. Our improved performance reinforces our confidence in the safe and responsible operation of the site," said Bossert.

Rickard described key laboratory animal studies, as well as 3M worker epidemiology studies, that support DuPont's position that there are no known adverse human health effects associated with C8. He also presented DuPont employee blood data to demonstrate the implausibility that residents in the communities surrounding the Washington Works site could have blood levels of C8 higher than Company employees.

-more-

"We know for a fact that there have been no observed adverse health effects among 3M and DuPont employees who have worked with C8 – people that we know have higher C8 blood levels than employees working outside the area of the plant where C8 is used and who live in various communities in West Virginia and Ohio," said Rickard.

At the briefing, DuPont also introduced recent updates to its C8inform.com website, including the report on emissions reductions, copies of communications between the company's leadership and state regulators, and copies of communications to Washington Works employees.

"We hope that members of the community will continue to seek out the facts about C8 as the public discussion continues," said Bossert. "We created the site to share information with the community in a convenient way and to respond to interests and concerns openly and honestly. That remains our goal."

# # #

Site contact:
Dawn Jackson
304-863-2513

EXHIBIT C-57

E. I. du Pont de Nemours and Company
Washington Works
Mail: P.O. Box 1217
Washington, WV 26181-1217

Contact: Robin Ollis
(304) 863-2513

DuPont Reiterates Position on PFOA
Refutes Claim that PFOA Causes Human Health Effects

Parkersburg, May 5 -- DuPont issued a statement today in response to claims that allege exposure to PFOA is responsible for elevated cancer rates among residents in the Parkersburg area. The claims were based on a "study" by Dr. James Dahlgren, a toxicologist retained by plaintiffs' lawyers. DuPont refutes this claim and questions the scientific validity of the "study."

"DuPont's position is very clear: PFOA is not a human carcinogen and there are no known health effects associated with PFOA," said Dr. Robert Rickard, DuPont lead toxicologist. "Dahlgren's claims are inaccurate and inconsistent with published scientific studies. In fact, the more we study PFOA, the more confident we are in our conclusions that PFOA is safe."

"Based on what we have seen, we question the scientific validity of the conclusion in the Dahlgren report. It is clear from the abstract of the paper, and the methodology reported at a recent meeting in Europe, that the analyses do not represent the scientific approach used by epidemiologists," stated Dr. Robin Leonard, principal epidemiologist for DuPont. "There is no indication that other factors impacting the health of populations were considered or analyzed. Based on our review of the limited information available, we believe that:

- Appropriate comparisons were not made using age-adjusted, standardized populations; this results in conclusions that are unfounded.
- Recruitment for the report was made through radio and television advertising; there is no evidence that it reflects an accurate representation of the community.
- The report purports to use DuPont data as a cancer prevalence rate comparison, yet DuPont did not report prevalence data. We reported 50 years of age-adjusted, cumulative 'incidence' data and rates. It is inappropriate to compare DuPont data with resident prevalence data.
- And finally, the report does not measure PFOA exposure at the individual level so no estimate of risk due to PFOA exposure can be made. The report focused only on occurrence of cancer and did not take into consideration any lifestyle or medical history, all of which were captured in the questionnaire given to the residents but were not analyzed."

The report itself was generated in the context of ongoing class action litigation filed against DuPont on behalf of residents who live near our Parkersburg site. It is an example of unscientific reporting and alarmist media coverage that does a disservice to our employees and the community in which they live.

Based on the information we have, we believe that the report does not support Dahlgren's statement that "there is clear evidence of health effects" associated with exposure to PFOA.

5/5/04

E. I. du Pont de Nemours and Company
Shipping: 8480 DuPont Rd
Washington, WV 26181

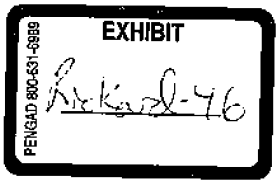


EXHIBIT C-58

DuPont Haskell Laboratory
for Health and Environmental Sciences
Elkton Road, P.O. Box 50
Newark, DE 19714-0050

March 9, 2005

Dr. Suhair Shallal
Designated Federal Officer
U.S. Environmental Protection Agency, Science Advisory Board (1400F)
Ariel Rios Building
1200 Pennsylvania Avenue, NW
Washington, D.C. 20460

Re: PFOA Risk Assessment Science Advisory Board

Dear Dr. Shallal:

On behalf of E. I. DuPont de Nemours and Company (DuPont), I want to thank you for the opportunity to provide additional comments to the PFOA Risk Assessment Science Advisory Board (SAB). A number of issues and recommendations were discussed in the February 22-23rd SAB review meetings, in particular, the need for additional data. With this submission, DuPont is informing the SAB of significant additional data that will be available very soon and providing clarification and perspective on a number of issues that were discussed during the SAB review.

The January 2005 EPA Draft Risk Assessment on PFOA reviewed data available through June 2004. EPA acknowledged that additional data were available and would be incorporated in future drafts. Although human health data were reviewed, the Draft Risk Assessment was based on results from laboratory studies and therefore, there were no specific charge questions for the SAB relative to the significance of human health data. The SAB clearly expressed a need for more human data and a desire to consider the relevance of human health data to the risk assessment. We concur and believe that health data on workers, the highest exposed population, are the most relevant data and deserve greater consideration in the final risk assessment.

Average serum levels in workers are 100–3000 times greater than the average serum level in the general population, and employee health data from five plant sites have been reported. DuPont has recently begun a comprehensive two-phase employee health study on PFOA at its Washington Works site in West Virginia. A report of the Phase 1 cross-sectional study of over 1000 employees (781 males; 243 females) is scheduled for May 2005, and results of the Phase 2 retrospective mortality study is expected by mid-year. In addition, investigators at the University of Pennsylvania are also conducting an independent, NIEHS-sponsored community exposure/health study. This study will examine a population living in Ohio around the Washington Works plant. The results of this study are expected later this year.

The SAB also suggested that studies to assess PFOA selectivity across the PPAR receptor class would be additive. In April of 2005, DuPont expects to report the results of a study investigating PFOA activation of PPARs and other nuclear receptors in a mouse-based cell line transfected

with human, rat and mouse receptors. PFOA activity was compared with dietary fatty acids and relevant positive controls. Preliminary results of this study confirm that PFOA is a PPAR-α agonist. Based on the EC_{50} in these assays relative to serum levels in the general population, PFOA is unlikely to elicit PPAR-α-mediated changes in the general population. Furthermore, PFOA showed weak or no activity against other human nuclear receptors known to play a role in lipid metabolism.

To date, no human health effects are known to be caused by PFOA exposure. The only potentially relevant association is a modest increase in some, but not all, serum lipid parameters in some of the highest exposed workers from the Phase I study noted above. It is unclear if this association is caused by PFOA exposure or is related to some other variable. DuPont is committed to investigate this issue further.

A more detailed description of the employee health study and the receptor study is provided in Attachment I. Full reports of both studies will be provided as soon as they are issued. Attachments I and II also provide additional clarification and perspectives on many of the issues discussed by the SAB.

In conclusion, DuPont believes that the weight-of-evidence indicates an overall lack of biological response to PFOA at exposure levels observed in the general population. Therefore, we believe that PFOA does not pose a risk to this population. The additional data from employee and community studies will provide important information to more fully assess the potential health effects of PFOA in higher exposed populations. Given the importance of this risk assessment and the near-term availability of these highly relevant data, it is strongly recommended that the SAB incorporate these studies into its review prior to finalizing any conclusions or recommendations.

DuPont recognizes that the presence of PFOA in human blood raises questions that should be addressed and is fully supportive of the EPA risk assessment process. DuPont is committed to objective and transparent research and looks forward to sharing the results of its studies as soon as available.

Sincerely,

Robert W. Rickard, Ph.D.
Science Director

RWR:jhh

Attachment I (17 pages)
Attachment II (23 pages)

CC: Charles M. Auer
Oscar Hernandez
Jennifer G. Seed

ATTACHMENT I

Comments on behalf of E. I. DuPont de Nemours and Company
to the PFOA Science Advisory Board

This document provides additional comment to the PFOA Science Advisory Board (SAB) on the following subjects:

1. Human Health Studies
2. Activation of Nuclear Receptors by PFOA and Naturally Occurring Fatty Acids
3. Mammary Gland Tumors in the 2-Year PFOA Study (Riker)
4. Pituitary Weight Changes in F1-Females in the Two-Generation Reproduction Study
5. Evaluation of PFOA for Immunotoxicity
6. Evaluation of PFOA for Neurotoxicity

Each subject is addressed in detail in the following discussion.

Gerald L. Kennedy, Jr., B.S.
Robin C. Leonard, Ph.D.
Nancy E. Everds, D.V.M.
Steven R. Frame, D.V.M., Ph.D.
Peter J. Gillies, Ph.D.
Scott E. Loveless, Ph.D.

1. Human Health Studies

No known health effects have been observed in an occupational setting due to exposure to PFOA. Several studies by the DuPont Company and 3M that have examined a range of outcomes including liver enzyme measurements, cancer incidence, all-cause mortality, and incidence of care frequency from health insurance data, have not indicated any health issues occurring as a result of exposure to PFOA (Fayerweather, 1981; Gilliland and Mandel, 1996; Olsen et al., 1998; Olsen et al., 2000; Olsen et al., 2003a; Olsen et al., 2003b).

a. DuPont Epidemiology Program

Data from the DuPont Epidemiology Program's most recent Washington Works, West Virginia plant site cancer incidence and mortality surveillance (DuPont, 2003a; DuPont 2003b), referenced in the risk assessment, have been misinterpreted in some discussions as representing PFOA-specific findings. It is important to note that the epidemiology surveillance program is a screening program for comparing mortality and cancer incidence at each DuPont manufacturing site with the mortality and cancer incidence of U.S. DuPont employees. These data cannot be used to make any conclusion as to PFOA causality for the following reasons:

- Results are plant-site specific, and not chemical specific
- They compare data from the plant employees to the US Dupont employee population, and not to the regional community
- Only about 25% of the Washington Works employees work with PFOA, thus precluding any conclusions about PFOA specifically

If potentially significant increases in any cause of death, or any incidence of cancer, are noted from a routine surveillance, further steps are taken to collect work and medical histories. It is not unusual to observe occasional increases in some parameters which require further follow-up. For example, a 1981 surveillance of the Washington Work site identified an increase Standardized Mortality Ratio (SMR) of myocardial infarction.

However, a more detailed follow-up investigation indicated that the increase was clearly not related to PFOA exposure, or to any other workplace chemical (DuPont, 1981).

b. Two-Phase, On-Going Study at DuPont Washington Works Site

A comprehensive two-phase PFOA study is currently in progress at the Washington Works site. Phase I is a cross-sectional survey of 1,025 workers (781 males; 243 females) at the plant that incorporates a biomarker of PFOA exposure (serum PFOA level) and clinical and questionnaire data from physical examination by occupational physicians. The primary objective of Phase I is to describe the relationship of serum PFOA to potential health outcome variables suggested by previous animal and worker studies, taking into account potential confounders and effect modifiers.

Preliminary results from this phase of the Washington Works study have indicated no association between PFOA serum levels and nearly all of the clinical laboratory (blood and urine) parameters examined. For example, no correlation was found between PFOA exposure and liver tests, the primary target organ in laboratory studies, nor in most other laboratory tests or any cancer markers, such as PSA. One exception was a modest increase in some serum lipid parameters in the subgroup of workers having PFOA levels greater than 1000 ppb. In contrast, no associations were seen between PFOA exposure and HDL cholesterol or C-reactive protein. Two other parameters, uric acid and iron, appeared increased in the serum of employees with the highest PFOA levels. Due to the cross-sectional nature of the Phase I design, the study data did not allow conclusions as to whether PFOA was or was not the cause of the changes in laboratory tests. Studies are being designed to further investigate these observations. The complete results of the current study are anticipated by May 2005.

Phase II is a retrospective cohort mortality study of all DuPont employees ever employed at the plant site. The study's primary objective is to compare observed deaths in the historical Washington Works population to the expected numbers of deaths based on five reference populations–all U.S. DuPont employees; State of West Virginia; Wood County, West Virginia; Washington County, Ohio; and the general U.S. population. The size of

the cohort, which covers 50 years of plant operations, combined with the availability of a specific biomarker of exposure (serum PFOA levels) that can be linked to specific job tasks, which can then be used to estimate past exposures. This makes this study very relevant to the understanding of PFOA and human health. Phase II results are scheduled to be complete and available by June 2005.

c. Study Sponsored by Plastics Europe

In addition to the ongoing PFOA worker studies, a collaborative cohort mortality study for tetrafluoroethylene (TFE) workers is being sponsored by Plastics Europe, an international trade organization (Bertazzi, 2004). Data have been collected for this effort at the Washington Works facility, as well as plants in Italy, Germany, England, and the Netherlands. This study will provide a detailed examination of mortality risk associated with specific exposure to several process chemicals–including PFOA–used for the manufacture of TFE.

d. NIEHS Sponsored Study

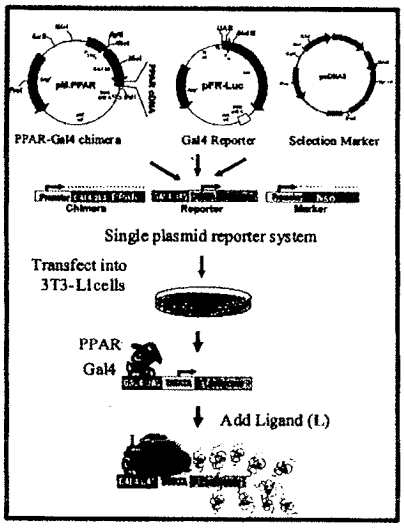
NIEHS is currently sponsoring a study in Washington County, Ohio, that is being conducted by investigators at the University of Pennsylvania. This study is designed to measure and investigate potential correlates of serum PFOA levels with several health endpoints and questionnaire responses from a random sample of the county population. These serum samples have been collected and will be analyzed by the summer of 2005.

2. Activation of Nuclear Receptors by PFOA and Naturally Occurring Fatty Acids

PFOA has been shown to be an agonist for the nuclear receptor, PPAR-α (Kennedy et al., 2004). Decreases in some lipid parameters are well-known effects of many PPAR-α agonists. Therefore, *in vitro* studies were conducted across multiple species, including humans, to (a) characterize the activity of linear PFOA on PPAR-α and other selected nuclear receptors involved in lipid metabolism; and (b) to compare the relative activity of PFOA to naturally occurring dietary fatty acids. These studies used a common testing platform of a murine-based cell line transfected with human, rat, and mouse receptors.

The analysis for the human receptors has been completed and demonstrates that:

- Naturally occurring dietary fatty acids were more potent agonists of PPAR-α than PFOA
- PFOA showed very weak or no agonism of PPAR-β, PPAR-γ, LXR-β or RXR-α



Human Nuclear Receptor	EC50 μM				
	Positive Control	PFOA	Octanoate	Linoleate	Linolenate
PPAR-α	8.6	45.2	40.8	18.4	8.6
PPAR-β	147.5	-	-	35.0	77.7
PPAR-γ	0.1	13.3	41.4	18.4	4.4
LXR-β	0.2	-	-	-	-
RXR-α	3.1	-	-	20.3	294.1

Positive Controls:

PPAR-α	Ciprofibrate
PPAR-β	Tetradecylthioacetic acid
PPAR-γ	Rosiglitazone
LXR-β	T0901317
RXR-α	Methoprene

- indicates no activity

In addition to PFOA, three fatty acids were tested in these studies; these were octanoate and two essential fatty acids, linoleate and linolenate. Given the structure of the ligands studied, the nuclear receptors chosen for these studies were PPAR-α, β, and γ; LXR-β; and RXR-α. Details of this assay were reported previously (Bility et al., 2004). The ligand-binding domains of the various nuclear receptors were fused to the DNA-binding domain of the yeast transcription factor Gal4 under the control of the SV40 promoter. This plasmid also encoded the UAS-firefly luciferase reporter under the control of the Gal4 DNA response element. Mouse 3T3-L1 cells were subsequently transfected with the plasmid DNA (see Figure). Test ligands were dissolved in DMSO and incubated with the transfected 3T3-L1 cells for 24 hours. Fold induction of normalized luciferase activity was calculated relative to control cells treated with the DMSO vehicle. Experiments were

run as three independent samples per treatment group. Dose-response curves were developed for each ligand and EC50 values calculated from best-fit lines, these values are reported in the table.

In terms of PPAR-α, PFOA presented as a full agonist, but with less potency than linoleate and linolenate; octanoate presented as an equipotent partial agonist. In terms of PPAR-γ, PFOA presented as a very weak partial agonist, as peak activation and potency were 10- and 133-fold less than Rosiglitazone. PFOA was inactive at doses up to 200 μM with respect to LXR-β and RXR-α.

In summary, under the conditions of this trans-activation assay, PFOA was less biologically active and more selective in its activation of nuclear receptors than naturally-occurring fatty acids. Studies are in progress to profile these same ligands against the rat and mouse receptors. Results of these studies are expected to be available in April of 2005.

3. Mammary Gland Tumors in the 2-Year PFOA Study

The incidence of mammary gland tumors seen in female rats fed PFOA in the 2-year study (Riker, 1987) is not considered to be causally related to PFOA ingestion based on the following:

- For benign tumors (adenoma and fibroadenoma):
    - There was no definitive dose-response, and there was no statistical difference in groups when data were properly analyzed
    - Tumor incidence was within the historical control values for this strain and supplier
- For adenocarcinomas, the high dose incidence was less than the control incidence and the incidence in the treated groups was not dose-related

In this study, the incidence of fibroadenomas of the mammary gland was 22, 42, and 48% at 0, 30, and 300 ppm in diet, respectively. The dose-response was such that there

was no clear incidence difference between the 30 and 300 ppm groups despite a 10-fold difference in dose. The authors concluded that this was not a treatment-related response.

Assessment of the mammary tumor response in this study included a comparison to historical control data from an outside laboratory. Some have questioned the use of such historical data. While the optimum historical data for a chronic study conducted at a given laboratory is derived from studies conducted at that same laboratory, valuable information can nonetheless be derived from interlaboratory data for a given species and strain. For example, the National Toxicology Program maintains a historical control database which includes animals of the same species and strain that are taken from studies conducted at different test facilities.

The value of interlaboratory data is further enhanced if the tumor type in question demonstrates similar incidences and ranges of incidences across multiple laboratories. Such is the case for mammary gland fibroadenomas in Sprague-Dawley rats. The incidence of fibroadenomas in 13 studies conducted at DuPont Haskell Laboratory ranged from 24 to 54% with a mean of 37% (Sykes, 1987). Similarly, historical control data from Charles River Laboratories, for a time period contemporary (1977-1985) to the 2-year study with PFOA, gives the average fibroadenoma incidence of 34% with a range of 15 – 58% among 11 studies conducted at different laboratories (Lang, undated). These data demonstrate that the incidence of mammary gland fibroadenoma in groups of untreated female Sprague-Dawley rats is often high and markedly variable irrespective of the laboratory from which the data are derived. Based on these considerations, the use of interlaboratory historical data for mammary tumors in Sprague-Dawley rats is entirely appropriate.

The laboratory conducting the two-year study in rats with PFOA (Riker Laboratories) did not have an adequate historical control database, as it was the only chronic study conducted at this laboratory at that time. Thus, control incidences from other laboratories, but derived from the same supplier of Sprague Dawley rats, were used to aid the assessment of mammary gland tumors in the two-year study. The incidences of

fibroadenomas in the PFOA-treated groups (42 and 48%, respectively) were within the range of historical incidences for this tumor type in both the Charles River and Haskell Laboratory historical databases. Consistent with these findings, the incidence of fibroadenomas in the PFOA-treated groups was not statistically different from the Haskell Laboratory historical control incidence ($p < 0.05$).

These data support the study authors' conclusion that the distribution of fibroadenomas in the PFOA study was a reflection of background incidence and was not related to PFOA treatment.

The SAB also discussed the findings of mammary gland adenocarcinoma in female rats in the two-year study. The incidences of adenocarcinoma were 7/46, 14/45, and 5/44 in the control, 30 ppm, and 300 ppm groups, respectively. The incidences of adenocarcinoma are not dose related and the incidence in the 300 ppm (high dose) group is actually lower than controls. Furthermore the incidence in the 30 ppm group (the group with the highest incidence of adenocarcinoma) is not statistically significant relative to controls by the Fisher's exact test ($p < 0.05$). This lack of statistical difference is true whether one uses the number of mammary glands evaluated as the denominator or the total number of animals examined (n=50/group). Therefore, the overwhelming weight of evidence indicates that mammary gland adenocarcinoma was not a treatment-related effect in this study.

To further clarify tumor findings in the two-year study in rats with PFOA, and in response to questions raised by the SAB, an independent pathology working group will be convened to peer-review tumor findings in mammary glands from this study and to assess causality. A report from this working group is expected by June of 2005. The relevance of mammary tumors, as well as other endpoints, is discussed in Attachment II.

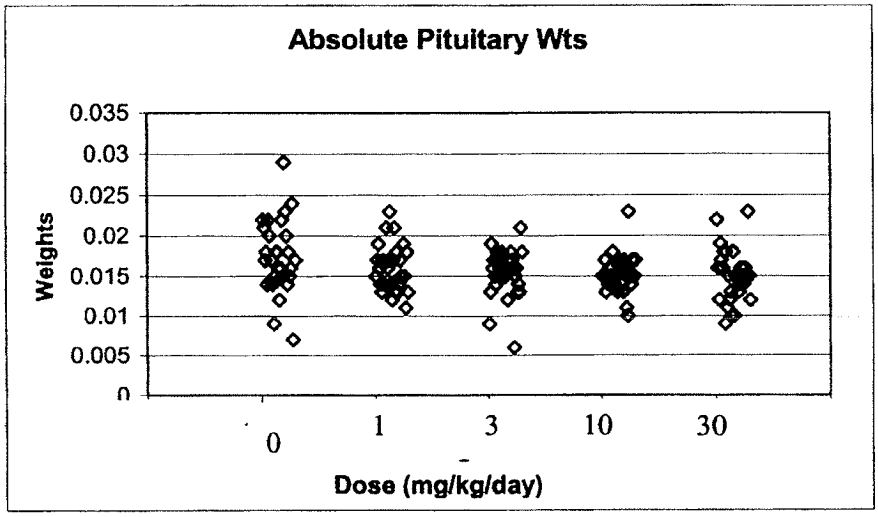
4. Pituitary Weight Changes in F1-Females in the Two-Generation Reproduction Study

In the two-generation reproduction study in rats with PFOA (Argus Research, 2002), statistically significant but minimal decreases in pituitary gland weights were observed in F_1-generation females administered 3 mg/kg/day and above. Weight-of-evidence considerations strongly indicate that these changes, though statistically significant, were spurious and not related to the administration of PFOA. This conclusion is based on the following:

- The statistically significant weight decrements were not dose related across a large (10-fold) range of doses
- Decreases were small compared to the variation in pituitary weight parameters observed within the control group population
- At the high dose, all individual pituitary weight parameters in F_1 females were within the range of values for individual control animals
- No pituitary weight changes were seen in the P-generation females
- No treatment-related microscopic changes occurred in the pituitary of female rats of either generation at any of the doses tested
- No treatment-related organ weight or microscopic changes were seen in male rats, clearly the more sensitive gender, in either generation at any of the doses tested

Statistically significant decreases in pituitary weight parameters in PFOA treated groups administered 3, 10, or 30 mg/kg/day in the two-generation reproduction study did not occur in a dose-related manner. For example, mean pituitary absolute weights in these three groups were identical (15 mg) despite the order-of-magnitude span in dosages. Furthermore, the decreases in pituitary weights relative to controls were very small. The mean absolute pituitary weights in the three groups administered 3 mg/kg/day and above were decreased 12% compared to the control group mean. However, the coefficient of variation for absolute pituitary weight within the control population was approximately 23%. The relatively large coefficient of variation noted among individual pituitary weights is consistent with the difficulty inherent in weighing very small organs.

The minimal nature of these pituitary weight changes is further underscored by a comparison of individual pituitary weights of animals in treated groups with those of the control group. Notably, all pituitary weight parameters (absolute weight and weight relative to both body and brain weight) in the F_1 female high-dose group were within the ranges of values for the within-study control group. A scatter-plot of individual absolute pituitary weights is given in the figure below.



Consistent with the minimal and likely spurious nature of the pituitary weight changes observed in some PFOA-treated F_1 female groups, no such statistically significant changes in pituitary weights were seen in the P-generation females. Furthermore, no treatment-related microscopic changes were seen in pituitaries of either the P- or F_1-generation female rats. More importantly, no pituitary effects–including organ weight or microscopic changes–were observed in either generation of male rats at any PFOA dose tested, including doses that clearly showed other evidence of toxicity. Male rats have consistently shown greater sensitivity to PFOA-associated toxicity, irrespective of the toxicity endpoint considered. This greater sensitivity is consistent with well-established pharmacokinetic differences between male and female rats. Thus, it is highly unlikely that true compound-related effects on the pituitary gland would be seen in females at

nontoxic doses as low as 3 mg/kg/day when no such pituitary effects were observed in males at a dose (30 mg/kg/day) that was 10-fold higher and overtly toxic.

In conclusion, the weight of evidence considerations noted above strongly indicate that the pituitary weight changes noted in some PFOA-treated F_1-generation female groups were spurious and unrelated to administration of the test material.

5. Evaluation of PFOA for Immunotoxicity

There is no definitive evidence that PFOA produces primary adverse effects on the immune system. This conclusion is based on the following considerations:

- Numerous toxicity studies in rodents and primates have shown no primary effects on the immune system organs or peripheral blood lymphocyte counts at doses that clearly produce systemic toxicity
- Reports by a single investigator of immune system effects in mice have several deficiencies which require further study before drawing conclusions as to their biological significance

Histopathological examination of immune system tissues (e.g., spleen, thymus, lymph nodes) is considered a sensitive endpoint to identify potential immunotoxicant hazards and should serve as part of a first tier evaluation (Basketter et al, 1994; Greaves, 2000). Organ weights of selected immune system organs are also recognized as valuable endpoints when evaluated in the context of all other clinical, clinical laboratory, and histopathology data from the study. In addition, routine analysis of hematology is also important in the initial evaluation of immunotoxicity (Ennulat et al, 2005, in press). Numerous multidose toxicity studies with PFOA in rats and monkeys have included assessment of these first tier end points for immunotoxicity. Based on these studies, PFOA does not produce primary toxicity to the immune system.

a. Immune system findings in toxicity studies in rats and monkeys

In a 90-day study, rats were fed PFOA at dietary concentrations ranging from 10-1000 ppm (IRDC, 1978a). There was no effect on peripheral blood lymphocyte counts. No effect was observed on spleen or thymus weight or histopathology. Similarly, in a two-year feeding study in rats fed PFOA at dietary concentrations of 30 or 300 ppm (Riker Laboratories, 1987) , no immunotoxicity was evident at either dietary concentration based on the absence of compound-related effects on peripheral blood lymphocyte counts, organ weight or histopathological changes in spleen, or histopathological changes in lymph nodes. More recently, an oral gavage 2-generation reproductive study (1, 3, 10, 30 mg/kg) was reported (Argus Research, 2002). Although clear evidence of systemic toxicity was seen in male rats based on body weight effects at the higher doses, no effects on spleen or thymus weights were observed at any dose in parental or F1 generations when organ weight was analyzed as a percentage of body weight (to account for the significant body weight decrements). In addition, no effects on spleen or thymus weight were observed in F1 or F2 pups. Thus, no effects were observed in offspring of PFOA-dosed dams at any of the doses tested.

In rhesus monkeys, PFOA administration did not affect peripheral blood lymphocyte counts or spleen or thymus weights at any dose (IRDC, 1978b) and no effects on lymphoid histopathology were observed at nonlethal levels (<30 mg/kg). In cynomologous monkeys fed 0, 2 or 20 mg/kg PFOA for 4 weeks or 0, 3, 10 or 20/30 mg/kg PFOA for 6 months, no compound-related microscopic changes were present in spleen, thymus and mesenteric lymph nodes at any dose, including doses that produced marked systemic toxicity (Covance Laboratory, 2001). In addition, there were no treatment-related changes in peripheral lymphocyte counts.

In summary, PFOA has been evaluated in numerous toxicity studies in rodents and monkeys. Most of these studies included assessment of peripheral blood lymphocyte counts, organ weights, and histopathological findings in immune system organs at doses that clearly produced systemic toxicity. However, no evidence of primary toxicity to the immune system has been observed following PFOA exposure.

b. Studies Evaluating Immunotoxicological Endpoints in Mice

Findings suggestive of immunotoxicity following exposure to PFOA are limited to several studies in mice reported by Yang and colleagues (Yang et al., 2000; Yang et al., 2001; Yang et al., 2002a; Yang et al., 2002b). Notably, most (but not all) of these studies evaluated immunotoxicity at a very high dietary concentration (0.02%) that produced marked systemic toxicity as evidenced by, for example, body weight loss of 17% after only 5 days of dosing. Nonspecific toxicity to the immune system secondary to marked systemic toxicity is a well-established phenomenon which is not addressed in these studies (Greaves, 2000). Also, these studies typically used very low numbers of animals (usually 4 mice/group) and, for some critical parameters measured, showed marked variability, even between similarly-treated controls. For example, relative liver weights in two different control groups (but groups used under the same study protocol) differed by 17% (Yang et al., 2000, Table 1a). A number of other observations suggest that further work is needed to understand the significance of the findings in this series of studies in mice:

- In one study, extremely high titers for anti-HRBC IgM antibody were reported in mice not immunized with HRBC; this raises concern about the specificity of the IgM antibody assay.
- In these studies, IgM antibody production as assessed by splenic plaque forming cells (PFC) were evaluated on the same day as serum specific antibody. However, for a given antigen, IgM antibody production as assessed by splenic PFC generally peaks one day earlier than serum IgM, as measured by ELISA. Based on the lack of data indicating time-to-peak antibody formation for this particular antigen and this strain of mice, any effect of treatment on IgM concentration cannot be evaluated.
- No histopathology of spleen, thymus or lymph nodes was performed. (Tier 1 endpoints).

In summary, based on the absence of primary target organ toxicity to the immune system across several toxicity studies in rats and monkeys, PFOA is not an immunotoxic

compound. Further work is necessary to clarify the results of studies in mice in which high doses of PFOA were reported to produce decrements in some immune parameters. Studies are being designed to clarify the immune system findings in mice reported by Yang et al.

6. Evaluation of PFOA for Neurotoxicity

Based on the absence of clinical signs or histological findings, exposure to PFOA is not associated with neurotoxicity.

No neurotoxicity has been detected in multiple toxicity studies by multiple routes of exposure in rats and monkeys. An increase in the incidence of ataxia in female rats from a 2-year feeding study was seen primarily in moribund rats toward the end of the study (Riker, 1987). An increase was not seen in male rats from this study or a second 2-year bioassay. Further, tissue distribution studies in rats show similar PFOA concentrations in the brain of males and females following oral dosing, indicating that a sex-specific response is unlikely. The body burden at steady state in males is considerably higher than that in females due to the ability of female rats to excrete PFOA in the urine. Hence it is again less likely that the female would be more responsive than the male.

In repeated exposure studies in rats and monkeys, the individual animals are routinely handled and evaluated for changes that may be indicative of nervous system dysfunction. Such evidence includes clinical signs of tremors, convulsions, gait/coordination abnormalities, lacrimation, salivation, excessive vocalization, changes in muscle tone, breathing rate, abnormal posture, arousal (activity level), polyuria, and diarrhea. In the battery of studies conducted on PFOA, a dose-related effect was not detected for any of these signs. In addition, morphological changes were not detected in the central or peripheral nervous system (including sciatic nerve, skeletal muscle, brain or spinal cord). Therefore, nervous system dysfunction has not been associated with PFOA exposure.

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ATTACHMENT II

(U.S. EPA Public Docket AR 226-1727)

Genotoxicity, Carcinogenicity, Developmental Effects and Reproductive Effects of Perfluorooctanoate: A Perspective from Available Animal and Human Studies

Prepared for the Association of Plastics Manufacturers of Europe and the Society of the Plastics Industry

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Introduction

This document will describe the experimental database for genotoxicity, carcinogenicity, developmental, and reproductive effects of perfluorooctanoate (PFOA) and will provide our current understanding of the potential relationship of these toxicological endpoints to man, as supported by studies of worker populations. In addition, it provides perspective on the relationship of these toxicological endpoints to human exposure and potential human health risk. PFOA and its salts are fully fluorinated organic compounds that are used as reactive intermediates or as processing aids and surfactants. A large toxicological and epidemiological database exists for PFOA. Most of the toxicological data have been developed using the ammonium salt of perfluorooctanoic acid (APFO); however, since APFO readily dissociates and is soluble in aqueous solution, the designation PFOA will be used throughout this document. The reader is referred to the U.S.E.P.A. document, "Revised Draft Hazard Assessment of Perfluorooctanoic Acid and its Salts" (U.S.E.P.A., 2002), for a detailed presentation of the toxicological and human-health databases for PFOA. Laboratory studies designed to identify potential health hazards of PFOA demonstrate that PFOA can produce effects in animal models. By contrast, the health effects observed in laboratory studies have not been observed in worker populations either under current or past exposure conditions. Therefore, we believe that PFOA does not present an unreasonable risk to human health at the levels encountered in the workplace.

Background on Worker Studies

Throughout this document, reference will be made to several worker studies. Studies in workers include cohorts from a PFOA production facility (Cottage Grove, MN) and two facilities that used PFOA in manufacturing processes (Decatur, AL and Antwerp, Belgium). The workers from the Cottage Grove facility are considered likely to have the highest potential for exposure since this facility manufactured PFOA since the 1940's and employees have been shown to have higher serum concentrations of PFOA than either Decatur or Antwerp plant populations. The Antwerp plant also manufactured PFOA but began in the mid 1970's. The Decatur facility routinely used PFOA but did not manufacture it until the late 1990's.and Antwerp plants are facilities that manufactured other fluorochemicals and routinely used PFOA. The types of studies performed include evaluations of mortality, medical surveillance, and episodes of care. The mortality studies examined observed versus expected causes of death in the study populations. Medical surveillance included standard worker health assessments as well as evaluations of biochemical parameters that had been affected in laboratory animal studies. An episodes-of-care study examined health insurance claims data. An episode of care is defined as a series of events all related to a particular health problem that exists continuously for a period of time.

Developmental Toxicity

The developmental toxicity of PFOA has been studied in rats and rabbits by the oral exposure route and in rats by the inhalation exposure route (Gortner, 1981; Gortner, 1982; Staples *et al.*, 1984). In those studies, pregnant animals were treated with graded doses/exposures of PFOA during organogenesis. Observations of the structural integrity of the fetuses was evaluated both

externally, internally, and by skeletal examination of the fetuses obtained prior to natural delivery. For one set of oral and inhalation studies in rats, dams were allowed to litter and pups were observed through the lactation period. These studies, as discussed below, allow the conclusion that maternal exposure to PFOA during organogenesis is not uniquely hazardous to the fetus or to preweaning rat pups.

The developmental study conducted in rats by Gortner (1981) was the first to be conducted with PFOA. In this study, maternal toxicity was observed at the highest dose (150 mg/kg) and consisted of group mean body weight reductions and mortality (3 of 22 dams). Reproductive organs were unaffected by treatment. Fetal examination did not reveal any increase in embryo-fetal toxicity or structural abnormalities that were attributable to PFOA treatment. Lens abnormalities, originally attributed to PFOA treatment, were found subsequently to be an artifact of the sectioning technique.

In another oral study, rats were given 100 mg PFOA/kg of body weight by gavage from gestation day 6 through 15 (Staples *et al.*, 1984). One group of 25 pregnant rats and their litters were examined on day 21 of gestation. Another group of 12 treated dams gave birth and the resulting pups were examined on day 35 post-partum. Maternal effects including death and decreased maternal body weight gain were seen in both groups. No developmental toxicity or abnormalities were seen in the fetuses, and offspring showed normal lactational viability.

By the inhalation route, groups of pregnant rats were exposed to concentrations of either 0.14, 1.2, 9.9, or 21 mg PFOA/m^3, 6 hrs/day from day 6 through 15 of gestation (Staples *et al.*, 1984). Exposure to the highest concentration resulted in the death of 3 of 12 rats with the remaining rats showing reduced weight gains and clinical signs including lethargy and chromodacyorrhea. Reduced weight gains were also seen in rats exposed to 9.9 mg/m^3. No effects were seen in those exposed to either 0.14 or 1.2 mg/m^3. Mean fetal body weights of surviving dams exposed to 21 mg/m^3 were reduced. There were no structural abnormalities in fetuses from any of the exposure groups that could be associated with PFOA exposure.

In a rabbit developmental study (Gortner, 1982), rabbits were given oral doses of either 1.5, 5, or 50 mg PFOA/kg from gestation day 6 through 18. The number of rabbits producing litters in this study was low in all groups, a fact that affects interpretation of the study. A reduction in maternal body weight gain was observed in rabbits given 50, but not 1.5 or 5 mg/kg. No other signs of response to PFOA were observed in the pregnant rabbits. Fetuses from all treatment groups were present in the expected numbers, were structurally normal, and weighed essentially the same as their untreated counterparts. No evidence of either embryotoxicity or teratogenicity was seen. An increase in the number of fetuses with the natural and stress-related variant of thirteenth ribs was noted. This latter finding is known to be quite variable (Christian, 1987), is not a malformation *per se*, and is not likely to be relevant to humans.

Regarding reproductive development, the multigeneration reproduction study with PFOA in rats showed delays in the age at preputial separation (mean = 3.7 days) in males and the age at vaginal opening (mean = 1.7 days) in females (York,2002). These delays are believed to be secondary to toxicity and do not represent a primary effect on organ development, as will be further discussed in the "Reproductive Effects" section that follows.

Reproductive Effects

A two-generation reproduction study in rats was conducted with PFOA (York, 2002). Rats were treated with oral gavage doses of either 1, 3, 10, or 30 mg/kg of body weight/day. In the parental rats, signs of toxicity were observed at all dose levels in the males and at 30 mg/kg in females. In males, body weight gain suppression was observed at all doses (except 1 mg/kg in the P_1 generation) along with organ weight changes (liver, kidney, and spleen). Female parental rats were relatively unaffected by treatment, with decreased kidney weights seen in P_1 females and decreased weight gains in F_1 females only at 30 mg/kg. There were no effects on any of the mating or fertility parameters in either generation. At 30 mg/kg, a number of effects in the offspring were observed including decreased pup weights, increased pup mortality (F_1 generation only), and delayed vaginal opening and preputial separation. These findings were not observed at any of the lower doses. Clearly, the effects observed in the two-generation reproduction study did not compromise the reproductive success (i.e., mating and fertility) of the rats at dosages of up to 30 mg/kg under the conditions of this study.

The two-generation reproduction study found decreased pup weights during lactation and increased pup mortality in the F_1 but not the F_2 generation. The increases in pup mortality occurring pre- and post-weaning at 30 mg/kg may be suggestive of the beginning of a dose-response curve. It is important to note that, while post-weaning mortality was not evaluated in the F_2 generation offspring (all F_2 offspring were necropsied at weaning), there were no effects on pre-weaning mortality in the F_2 offspring (pre-weaning mortality was increased in F_1 pups, but was not statistically significant). In addition, there were no effects on pup weights in F_2 generation offspring through weaning.

The increased incidence of pup mortality at 30 mg/kg is most likely a result of a general failure to thrive of the offspring, suggesting a compromised nutritional status of the offspring at pre- and/or post-weaning as reflected by reduced body weight. In support of this hypothesis, eleven of the thirteen F_1 offspring that died post-weaning died before post-weaning day eight, and these included the nine lightest pups. Although not statistically significant at all time points, pup weights were consistently decreased throughout the lactation period (90, 90, 89, 92, and 95% of control on postnatal days 1, 5, 8, 15, and 22, respectively). These effects have also been observed in reproduction studies performed with other peroxisome proliferators such as gemfibrozil, RMI 14,514, and hydrochlorofluorocarbon 123 (HCFC-123) (Fitzgerald *et al.*, 1987; Gibson *et al.*, 1981; Malinverno *et al.*, 1996). It seems likely that the compromised nutritional status of some offspring is responsible for the increased pup mortality observed in the two-generation reproduction study with PFOA.

The data from this study, discussed in more detail below, shows delayed age at preputial separation in males (mean = 3.7 days) and delayed age at vaginal opening (mean = 1.7 days) in females in the F_1 offspring. The delays in sexual maturation may have been the result of delayed growth of the F_1 offspring. As noted earlier, pup weights were consistently decreased throughout the lactation period. While the body weights of the F_1 generation offspring were similar to the controls at the time of sexual maturation, it is plausible that the delayed growth that was

observed early in lactation may have contributed to the delays that were observed in sexual maturation of the F_1 offspring.

Decreased body weights can result in non-specific delays in puberty (Carney *et al.*, 1998; Glass *et al.*, 1976; Glass & Swerdloff, 1980; Kennedy and Mitra, 1963; Marty *et al.*, 1999, 2001a, 2001b, 2001c; Ronnekleiv *et al.*, 1978; Stoker *et al.*, 2000a; 2000b; Widdowson & McCance, 1960). In a recent report by Lewis and co-workers (2002), variability of sexual maturation data was evaluated in control populations of Sprague-Dawley rats. They found that the typical variability among control groups was approximately two days, a finding that was also consistent with the typical variability in age at sexual maturation reported by others (Ashby & Lefevre, 2000; Clark, 1999; Marty *et al.*, 1999; Stoker *et al.*, 2000b). Since non-specific effects on body weight can cause general delays in sexual maturation, interpreting delays in sexual maturation can be problematic in studies where generalized delays in growth occur, such as those that were observed in the current study of PFOA. Clearly, PFOA do not compromise reproductive success (i.e., mating and fertility) in rats at dosages of up to 30 mg/kg.

In summary, in the two-generation reproduction study with PFOA, paternal toxicity (P_1 and F_1) was observed at all dose levels (1, 3, 10, and 30 mg/kg) and minimal maternal toxicity was observed at 30 mg/kg. While several possible reproductive/developmental effects were observed (i.e., decreased pup weights, increased pup mortality, and delayed sexual maturation in F_1 offspring), the reproductive success of the rats was not compromised. Notably, the overall results of the first and second generation appear to be similar in that there was no apparent increase in adverse outcome(s) in the second generation. The effects that were observed could be suggestive of reproductive and/or developmental effects or they could be due to general delays in growth. Unknown mechanisms may be contributing to the effects that were observed at 30 mg/kg. At dosages of $\leq$ 10 mg/kg, no reproductive or developmental parameters were affected, while parental males showed clear signs of toxicity. The no-observed-adverse-effect-level (NOAEL) for reproduction in the two-generation reproduction study was 10 mg/kg, while the NOAEL for general toxicity would be $<$ 1 mg/kg for the male parental animals and 10 mg/kg for the female parental animals. The effects that were observed with PFOA in the two-generation reproduction study are consistent with those observed in studies with other peroxisome-proliferating compounds (Fitzgerald *et al.*, 1987; Gibson *et al.*, 1981; Malinverno *et al.*, 1996).

Human Experience with Respect to Development and Reproduction

An episodes-of-care study (Olsen *et al.*, 2001b) at the 3M Decatur plant site examined reproductive outcomes associated with fluorochemical exposure (which includes potential PFOA exposure). Regarding pregnancy and its potential complications, there were 40 episodes of care reported in 13 female employees in the fluorochemical plant (44.7 expected) compared to 23 episodes of care (26.3 expected) reported in eight female employees in the film plant (a non-fluorochemical plant at the same site as the Decatur fluorochemical plant) between 1993 and 1998. This resulted in an episodes of care risk ratio of 1.0 (95% CI 0.6-1.8). The total number of female employees was 122 and 101 in the chemical and film plants, respectively. The episodes-of-care risk ratios for congenital anomalies (1.0, 95% CI 0.6-1.8) as well as perinatal disorders (0.2, 95% CI 0.0 - 2.4) were also comparable between employees in the fluorochemical

and film plants. There is no evidence from this study to suggest increases in reproductive and developmental effects associated with exposure to fluorochemicals including PFOA.

Hormones

The association of PFOA serum and hormone concentrations in workers has been studied at three production facilities (Cottage Grove, Decatur and Antwerp). The episodes-of-care study conducted only at the Decatur facility also allowed observation of episodes of care that may relate to hormonal status. Two cross-sectional studies of 111 and 80 Cottage Grove male fluorochemical production workers were conducted and measured their serum PFOA concentrations in relation to the concentrations of several hormones (testosterone, estradiol, LH, FSH, DHEAS, TSH, cortisol and sex hormone-binding globulin) (Olsen *et al.*, 1998). PFOA serum concentration was not associated with changes in hormone concentrations. Although a 10% increase in mean estradiol level was observed among employees who had the highest levels of serum PFOA, this association was confounded by body mass index and was likely not due to PFOA exposure. Further, an analysis of thyroid hormone levels in 3M Antwerp and Decatur workers did not show substantial changes in TSH, T4, free T4, T3 or thyroid hormone binding globulin associated with serum PFOA concentrations (Olsen *et al.*, 2003b). The risk ratio for disorders of the thyroid in the episodes-of-care study was comparable between Decatur fluorochemical and film plant workers (1.1, 95% CI 0.6-1.8) (Olsen *et al.*, 2001b). In addition to these human observations, a six-month oral toxicity study in male cynomolgus monkeys did not produce significant changes in either sex hormones or thyroid hormones (Butenhoff *et al.*, 2002). Therefore, there is no observed association of PFOA exposure with changes in hormone levels in man or monkeys.

Genotoxicity

The weight of evidence from studies evaluating the genotoxicity of PFOA indicates that PFOA is not genotoxic. These studies include evaluation of mutagenicity, clastogenicity and cell transformation.

PFOA has not shown a potential to effect DNA point mutations or recombinations. PFOA has shown a lack of activity in bacterial reverse mutation assays including *Salmonella typhimurium* and *Escherechia coli* strains and in yeast recombination assays (*Saccharomyces cerevisiae*) in the absence and the presence of metabolic activation (Litton, 1978; Hazleton, 1995a, 1996a). Similarly, in the Chinese hamster ovary (CHO) forward mutation assay, PFOA did not induce a statistically significant increase in the number of mutant colonies in the treated cells (Toxicon, 2002).

Chromosomal aberrations were assessed in human lymphocytes and CHO cells. PFOA did not induce significant increases in the numbers of chromosomal aberrations in human lymphocytes (Hazleton, 1996b; NOTOX, 2000). When tested in CHO cells, significant cytotoxicity was observed at the highest doses tested, and these doses also increased chromosomal aberrations.

(Hazleton, 1996c, 1996d). In view of the high toxicity, the biological significance of this positive response is questionable.

PFOA did not induce a significant increase in bone marrow polychromatic erythrocytes after oral administration to mice (Hazleton, 1995b, 1996e). There was no evidence of cell transformation using the C3H 10T1/2 cell line observed at any of the dose levels tested (Stone, 1981). The genotoxicity profile for PFOA indicates a lack of activity in a range of test systems and endpoints.

Peroxisome Proliferation

PFOA is a peroxisome proliferator (PP) in numerous studies and belongs to a widening group of substances including plasticizers and hypolipidemic drugs that are known to be PPs (Ikeda *et al.*, 1985; Just *et al.*, 1989; Pastoor *et al.*, 1987; Cook *et al.*, 1992, 1994; Biegel *et al.*, 1995, 2001).

The liver is a primary target organ for both short-term and chronic effects of PFOA in rats (Griffith & Long, 1980; Olson & Anderssen, 1983; Kennedy, 1985; Pastoor *et al.*, 1987) and cynomolgus monkeys (Butenhoff *et al.*, 2002). The increased liver weight does not appear to be a result of hepatocellular hyperplasia (no increase in nuclear DNA) and has been variously attributed to increases in peroxisomes, endoplasmic reticulum and mitochondria (Ikeda *et al.*, 1985; Pastoor *et al.*, 1987; Butenhoff *et al.*, 2002; Berthiaume & Wallace, 2002; Biegel *et al.*, 2001). PFOA has been shown to activate the PPARα receptor (Maloney & Waxman, 1999). Higher doses lead to liver degeneration and necrosis and the appearance in the serum of enzymes reflecting liver damage.

Treatment of rodents with PPs initiates a characteristic sequence of morphological and biochemical events in the liver and to a lesser extent the kidney. These events include marked hepatocellular hypertrophy due to an increase in number and size of peroxisomes, large increases in peroxisomal fatty acid β-oxidation, an obvious swelling and proliferation of the mitochondria and endoplasmic reticulum, increased cytochrome P-450-mediated ω-hydroxylation of lauric acid, and various changes in lipid metabolism (Ikeda *et al.*, 1985; Pastoor *et al.*, 1987; Berthiaume & Wallace, 2002). This response is initiated by the activation of the nuclear receptor, PPARα (Green, 1995; Ashby *et al.*, 1994; Lake, 1995). PPARα is a steroid hormone receptor able to increase the transcription rate of responsive genes and is the major mediator of PP in rodent liver. The critical role of PPARα in PP in mice has recently been clearly established. PPARα-null mice do not show the typical PP-mediated responses or signs of hepatic hyperplasia or neoplasia (adenomas or carcinomas) in chronic studies with PPs (Peters *et al.*, 1997; Ward *et al.*, 1998). Long-term exposure of rodents to PPs characteristically results in an increased incidence of liver tumors (Doull *et al.*, 1999; IARC, 1995).

There are differences in the effects exerted by different PPs. Pronounced species differences have been reported following treatment of animals with PPs *in vivo* and have been observed in hepatocyte cultures *in vitro* (Ashby *et al.*, 1994; IARC, 1995; Bentley *et al.*, 1993; Elcombe *et al.*, 1997; Lake, 1995; Maloney & Waxman, 1999). Rats and mice are highly, perhaps uniquely, responsive to the effects of PPs; whereas, Syrian hamsters exhibit an intermediate response and guinea pigs seem to be practically nonresponsive, as are primates - including both Old World and

New World (e.g., marmoset) species, and humans (Bentley *et al.*, 1993; Pugh *et al.*, 2000; Butenhoff *et al.*, 2002; Tucker & Orton, 1993; Graham *et al.*, 1994).

A large number of humans have been treated for relatively long periods of time with hypolipidemic drugs that are potent PPs in rodents. No significant changes in the peroxisome number or volume occur in humans taking substantial doses of these drugs for extended periods of time (up to 3 years) (Ashby *et al.*, 1994). Two human epidemiology studies showed no indication of an increase in cancer associated with long-term human exposure (ranging up to eight years) to hypolipidemic drugs (Ashby *et al.*, 1994).

Rodents are poor models for human risk assessment with respect to liver effects observed with PPs. The reason for the non-responsiveness of humans to PPs is not yet fully understood; although, research shows differences in amount and expression of PPARα between humans and rodents (Cattley *et al.*, 1998; Palmer *et al.*, 1998).

Induction of liver, testicular Leydig cell and pancreatic acinar cell tumors is a common finding for PPs (Cook *et al.*, 1999). In chronic bioassays in rats, Cook *et al.* (1999) reported that 7 out of 11 PPs induced all three tumor types (Cook *et al.*,1999), and 10 of the 11 PPs produced liver and Leydig cell tumors (Cook *et al.*, 1999).

Cancer

The oncogenicity of PFOA has been investigated in two separate two-year feeding studies in rats. PFOA was found to increase the incidence of three tumor types (liver, Leydig cell, and pancreatic acinar cell tumors-Riker,1983, Biegel *et al.*, 2001). In the following discussion, each tumor type will be discussed in turn.

Hepatocellular Adenoma

In a chronic dietary study conducted with 156 male Sprague Dawley rats fed diets containing 300 ppm PFOA for two years (Biegel *et al.*, 2001), histopathological evaluation revealed PFOA-related increases in hepatocellular adenoma. Hepatocellular adenoma occurred at an incidence of 13 % (10/76) as compared to 3 % (2/80) and 1% (1/79) in *ad libitum* and pair-fed controls, respectively.

These liver tumors are believed to have resulted from peroxisome proliferation. Evidence for this comes from the measurement of hepatocellular peroxisome proliferation at three-month intervals during the study. Increased liver weights and hepatic ß-oxidation activity were observed in the PFOA-treated rats at all time points; however, PFOA did not significantly increase hepatic cell proliferation. It is generally agreed that liver tumors in rats produced by PPs are unlikely to be relevant to humans.

Human Experience with Regard to PFOA and Liver Toxicity

Several worker studies investigated the possible association between either liver cancer or liver-related disease with PFOA exposure and have shown no association. Exposures to PFOA in

these workers, as measured by serum PFOA concentration starting in 1993, ranged from less than 1 to 114 ppm (Olsen *et al.*, 2000, 2001a, 2001c, 2003a, 2003b). PFOA was not measured routinely prior to 1993 because a total organofluorine method was used. Past serum PFOA concentrations in workers may have been higher.

Epidemiological assessments of liver cancer deaths among 3M workers with potential exposure to PFOA have not shown significantly increased Standardized Mortality Ratios (SMRs) for liver cancer; although, very few deaths from liver cancer were expected. Among 182 workers identified with definite PFOA exposure at 3M's Cottage Grove plant, there were no deaths related to liver cancer or cirrhosis of the liver during a 50-year time period (0.3 and 1.2 expected, respectively) (Alexander, 2001a). Among 1,491 workers with probable PFOA exposure, there was one liver cancer death compared to 2.0 expected (SMR = 0.5, 95% CI 0.0 - 2.0) and 6 deaths attributable to cirrhosis of the liver (6.4 expected, SMR = 1.0, 95% CI 0.4-2.1).

At 3M's Decatur plant, PFOA has been used as an emulsifier in fluoropolymer production and has also been a residual by-product of perfluorooctanesulfonyl fluoride production. PFOA production did not occur until the late 1990's. Employee serum PFOA concentrations have ranged up to 13 ppm in sampling conducted in 1998 and 2000. In this population, there were two liver cancer deaths observed compared to 0.7 expected (SMR = 3.1, 95% CI 0.4-11.1) during a 38-year study period (1961-1998) of 1,065 workers (Alexander, 2001b). It is unlikely that these observations represent a response to PFOA.

Analysis of episodes of care (health claims data) over a six-year interval (1993-1998) of a subset of these Decatur workers (n = 652) did not show differences in reported disorders of the liver (cirrhosis and hepatitis) between this Decatur fluorochemical workforce and a comparison non-exposed workforce (Decatur film plant employees) (Olsen *et al.*, 2001b). There was a nonsignificantly increased risk ratio (1.6, 95% CI 0.8-2.9) of episodes of care of disorders of the biliary tract reported in 13 individuals in the fluorochemical plant (N = 652). This episodes of care risk ratio increased to 2.6 (95% CI 1.2-5.5) when restricted to the 211 fluorochemical workers with $\geq$ 10 years work experience (based on eight individuals' health claims data). An episodes of care study has not been done for Cottage Grove or Antwerp fluorochemical production workers.

Hepatic clinical chemistry test results have been reported in a series of cross-sectional assessments of medical surveillance examinations for both the Cottage Grove and Decatur employee populations as well as the fluorochemical production workforce located in Antwerp (Gilliland & Mandel, 1996; Olsen *et al.*, 2000; 2003b). None of these study populations have had changes in hepatic enzyme assays or bilirubin analyses that could be associated with measured serum PFOA concentrations after adjusting for potential confounding factors including body-mass index and alcohol consumption. Serum PFOA concentrations in 3M Antwerp workers were approximately half of those measured in the Decatur workforce (Olsen *et al.*, 2001a, 2001c, 2003b).

Liver Tumor Summary

In summary, the lack of indications of increased risk of liver disease in 3M workers with exposure to PFOA suggests that the exposures encountered by non-occupationally exposed individuals should present a low risk of liver disease and, by extension, liver cancer. The lack of genotoxicity observed in genotoxicity assays and the increase in peroxisome proliferation observed in the lifetime dietary study in rats suggests a potential mechanism for the increase in hepatocellular adenoma in rats. If peroxisome proliferation is involved in the etiology of the hepatocellular adenoma observed in rats, the risk of hepatocellular adenoma developing in exposed humans is expected to be quite low due to the much lower-degree of response to PPARα agonists in human liver.

Leydig Cell Tumors

Two chronic studies in Sprague Dawley rats have shown increases in hyperplasia and benign tumors (adenoma) of testicular Leydig cells. In the first study (Riker, 1983), the incidence of Leydig cell adenomas was 0/50, 3/50, and 7/50 at dosages of 0, 30, and 300 ppm PFOA, respectively. A second study by DuPont included numerous mechanistic endpoints (i.e., cell proliferation, hepatic enzyme measurements, hormone measurements) and was specifically designed to evaluate the mechanism of Leydig cell tumor induction (Biegel *et al.*, 2001). In this study, PFOA was administered at 0, 0-pair-fed, or 300 ppm PFOA to male rats. There was a increase in the incidence of Leydig cell hyperplasia and adenomas, with adenoma incidences of 0/80, 2/78, and 8/76 in the 0, 0-pair-fed, or 300 ppm PFOA group, respectively (Biegel *et al.*, 2001).

Experimental evidence for the mechanism of PFOA-induced Leydig cell tumor formation, while not conclusive, tends to support the hypothesis that a sustained increase in estradiol within the testes may be responsible for the increased incidence of Leydig cell tumors in male Sprague Dawley rats (Cook *et al.*, 1992; Biegel *et al.*, 1995; Liu *et al.*, 1996a, 1996b). The extent to which this effect may be linked to PPARα activation is not clear. Other PPs (DEHP and clofibrate) have been shown to increase serum estradiol concentrations in male rats (Eagon *et al.*, 1994; Rao *et al.*, 1984), and several PPs (e.g., clofibrate, DEHP, gemfibrozil, dibutyl phthalate, and Wyeth 14,643) have been shown to reduce estradiol metabolism, resulting in an increase in circulating levels of estradiol (Corton *et al.*, 1997; Eagon *et al.*, 1994; Fan *et al.*, 1998; Rao *et al.*, 1984). This pattern of hormonal alteration has also been observed *in vitro*, where 10 of 11 peroxisome proliferators evaluated increased estradiol levels, and 11 of these PPs decreased testosterone levels (Liu *et al.*, 1996a, 1996b). While most PPs may increase estradiol in rats, the direct association of elevated estradiol with the production of Leydig cell tumors remains to be demonstrated. There are seven proposed mechanisms for Leydig cell tumorigenesis in rodents, all of which disrupt the hormonal milieu within the testes (Clegg *et al.*, 1997; Cook *et al.*, 1999). The attribution of sustained estradiol increase as part of the response to PPARα activation and as the operative mechanism for PFOA-induced Leydig cell tumors as well as the relevance of these tumors to humans will require additional research.

Human Experience with Testicular Tumors

Testicular cancer is most commonly diagnosed under the age of 40 in humans (Schottenfeld, 1996). Ninety-five percent of neoplasms of the testes arise from germinal cells and are divided

clinically into the seminoma and a variety of pure and mixed types of nonseminomatous tumors. Non-germinal neoplasms constitute 5% of testicular tumors with approximately half of these being histologically classified as Leydig cell tumors. Mortality data do not adequately explain occupational risk for testicular cancer because of the high five-year survivability rates for testicular cancer (> 95% survival). Thus, it is not unexpected that there has been only one death attributable to testicular cancer among the 3M Cottage Grove fluorochemical production workers (0.4 expected) during a 50-year study period (Alexander *et al.*, 2001a) and no deaths due to testicular cancer observed among the Decatur occupational population (0.2 expected) in a 38-year study period (Alexander *et al.*, 2001b). Analysis of episodes of care among the Decatur population from 1993-1998 did find two individuals with health claims data coded to testicular cancer (0.6 expected) (Olsen *et al.*, 2001b). One of these two workers had ≥ 10 years of work experience in the fluorochemical plant.

As noted previously, there are no direct associations of PFOA exposure with changes in sex hormones. A 10% increase in mean estradiol level observed among employees who had the highest levels of serum PFOA was confounded by body mass index and likely was not due to PFOA exposure (Olsen *et al.*, 1998).

Testicular Tumor Summary

Although Leydig cell tumors have been observed in two cancer studies in rats, the occurrence of this tumor type in humans is rare. There is currently no evidence that a relationship between PFOA exposure and increased testicular cancer risk exists in humans. In addition, no hormonal changes that may be mechanistically related to testicular cancer have been observed in monkeys or humans with PFOA exposure.

Pancreatic Acinar Cell Tumors

Male Sprague Dawley rats fed diets containing 300 ppm PFOA for two years (Biegel *et al.*, 2001), exhibited an increase in pancreatic acinar cell adenoma and combined pancreatic acinar cell adenoma/carcinoma. Acinar cell adenoma incidence was 9 %, 0%, 1% in PFOA-treated rats, *ad libitum* fed controls, and pair-fed controls, respectively. A prior two-year dietary bioassay in male and female Sprague Dawley rats at 30 and 300 ppm PFOA did not result in an increase in pancreatic tumors (Riker, 1983); although, a subsequent pathology peer review has noted the presence of hyperplastic foci.

Pancreatic acinar cell tumors (Reddy & Rao, 1977) are often observed following chronic exposure of rodents to other PPs. The mechanism by which PFOA and some other PPs induce these tumors is not well understood. The development of these tumors is known to be modified and/or mediated by several factors such as steroid hormone levels, growth factors such as cholecystokinin (CCK) and dietary fat (Obourn *et al.*, 1997). Biegel *et al.*, (2001) have proposed that PFOA and other PPs could increase the fat content in the gut and stimulate CCK release that, in turn, could lead eventually to hyperplasia in the pancreatic acinar cells. It must be concluded that, at the present time, this is a speculative mechanism that is not supported by experimental evidence for PFOA (Biegel *et al.*, 2001; Butenhoff *et al.*, 2002) and its applicability to humans is uncertain (Gavin *et al.*, 1996, 1997; Cattley *et al.*, 1998; Pandol, 1998). Pancreatic

acinar cell adenomas are rare in humans (Anderson *et al.*, 1996) and when considering the relevance of this rat tumor data with regard to human health risk, the non-genotoxic mechanism (with a likely threshold), and the relatively low exposure in humans should be taken into account.

Human Experience with Pancreatic Disease

The pancreatic acinar cell tumors observed in PFOA-treated rats (Biegel *et al.*, 2001) are not commonly diagnosed in humans. Among the Cottage Grove workforce with definite PFOA exposure (n = 182), there was one death reported for pancreatic cancer compared to 0.8 expected (SMR = 1.3, 95% CI 0.0-7.4) (Alexander *et al.*, 2001a). Employees (n = 1,491) defined with probable PFOA exposure had six deaths attributable to pancreatic cancer compared to 4.8 expected (SMR = 1.4, 95% CI 0.5 - 2.7). These pancreatic cancers were likely to have been of ductular origin rather than acinar. At the 3M Decatur manufacturing site there were no deaths attributable to pancreatic cancer among the 1,065 employees with one expected (Alexander *et al.*, 2001b). One episode of care for pancreatic cancer has been reported (Olsen *et al.*, 2001b). Although the episodes of care risk ratio for acute pancreatitis was increased (2.6, 95% CI 0.6-15.8) among the fluorochemical production workforce, this effect is difficult to interpret because it is based on six health claims from just one employee.

Because a sustained elevation of CCK has been suggested as a potential mechanism for pancreatic cancer, plasma CCK levels were assayed in 74 Cottage Grove PFOA production workers participating in medical surveillance examinations in 1997 (Olsen *et al.*, 2000). CCK values (mean 28.5 pg/ml, SD 17.1, median 22.7 pg/ml, range 8.8-86.7 pg/ml) approximated the assay's reference range (up to 80 pg/ml) and were negatively, not positively, associated with employees' serum PFOA concentrations.

Pancreatic Tumor Summary

PFOA was associated with an increase in acinar cell tumors of the pancreas in rats in one of two separate two-year bioassays. This tumor type is rare in humans, and there is no epidemiological evidence for a relationship between PFOA exposure and pancreatic cancer. The relevance of acinar cell tumors of the pancreas in rats to human pancreatic cancer risk is uncertain.

Mammary Gland Tumors

In the 3M-cancer study with PFOA in Sprague Dawley rats (Riker, 1983), the incidence of fibroadenomas of the mammary gland apparently was increased in female rats (22%, 42%, and 48% at 0, 30, and 300 ppm in diet, respectively). There was no apparent difference in incidence over a ten-fold dose range. The authors of this study concluded that the mammary tumor data did not reflect an effect of PFOA.

The laboratory conducting the study, Riker Pharmaceuticals, did not have an adequate historical control database. However, untreated control rats (same strain and supplier) from 13 chronic toxicity/oncogenicity studies conducted at Haskell Laboratory from 1984-87 provided 947

control rats, which were on test for at least one year (scheduled sacrifice at two years). Charles River, the supplier, also maintains a control database.

Statistical evaluation of the incidence of fibroadenomas in the PFOA-treated groups versus the Haskell Laboratory historical controls was not significant ($p = 0.3$). The incidence of fibroadenomas in the 13 reference Haskell laboratory studies ranged from 24 to 54% with a mean of 37%. In the PFOA study, the control group incidence lies just below and the test group incidences lie near the top of the control range. The incidences in the PFOA-treated groups (42 and 48%) are similar to the average of the Haskell Laboratory historical control groups (37%).

Historical control data posted on the Charles River Laboratories Web-Site, gives the average fibroadenoma incidence of 41% with a range among 24 studies of 13 - 61%. These data further support the study authors' conclusion that the distribution of fibroadenomas in the PFOA study were a reflection of background incidence and were not related to PFOA treatment.

When all mammary tumors of epithelial origin in this study are combined, there is no statistically significant increase in total tumors. Mammary tumors in rats present as a continuum from benign to malignant. In composition. They range from tumors of primarily epithelial cells to various degrees of connective tissue involvement. From a biological perspective, both adenomas and fibroadenomas are classified as benign fibroepithelial tumors, and, when combined for the PFOA study is not statistically increased. Similarly, there is no biological difference between the terms adenocarcinoma and carcinoma. The data for total malignant tumors shows a lower incidence of malignant tumors in the high-dose compared to the control animals (17, 31, 11% in the 0, 30, 300 ppm groups).

Human Experience with Breast Cancer

The available human data do not suggest an increased breast cancer risk. There have been no breast cancer deaths observed among Cottage Grove workers identified with definite PFOA exposure (0.2 expected) and two breast cancer deaths observed among those with probable PFOA exposure (3.6 expected, SMR = 0.6, 95% CI 0.1 - 2.0) (Alexander, 2001a). There have been no breast cancer deaths in the Decatur fluorochemical production workforce (0.9 expected) (Alexander, 2001b). There were two episodes of care for breast cancer (3.5 expected) among a subset of the Decatur fluorochemical production workforce compared to zero episodes of care in the comparison film plant employee population (4.0 expected) (Olsen *et al.*, 2001b). One of these individuals had worked $\geq$ 10 years. As for benign neoplasms of the breast, the risk ratio was 1.1 (0.4-2.8) based on nine individual episodes of care in the Decatur fluorochemical plant and ten individual episodes of care in the film plant. Non-malignant disorders of the breast were slightly higher among Decatur fluorochemical female employees as the episodes of care risk ratio was 1.6 (95% CI 0.9-2.9) based on 28 individual episodes of care in the chemical plant and 19 individual episodes of care in the film plant. The majority of these episodes of care were identified as fibrocystic disease.

Mammary Tumor Summary

In summary, the tumors seen in the mammary glands of rats fed PFOA reflect background incidence.

Prostate Tumors

An epidemiological investigation of the Cottage Grove chemical division workforce associated prostate cancer mortality with employment duration in perfluorochemical production activities (Gilliland, 1992; Gilliland & Mandel, 1993). Specifically ≥ 10 years of employment was associated with a 3.3 fold increase (95% CI 1.0 -10.6) in prostate cancer mortality relative to workers not employed in the chemical division. A major limitation of this investigation, with regard to evaluating the potential effects of PFOA exposure, was the lack of job and department specificity in the duration of employment analyses. Only one Cottage Grove employee had worked directly in the PFOA production building (Olsen *et al.*, 1998). Alexander (2001a) addressed this limitation by computerizing all work history records of Cottage Grove employees with at least one year of cumulative employment and constructing a calendar year, job- and department- specific exposure matrix from this computerized database. Alexander (2001a) did not find prostate cancer mortality associated with duration of employment among those Cottage Grove employees with definite or possible exposure to PFOA (cases observed/expected): 0 - < 1 year (0/0.1), 1 - < 5 years (2/1.4), 5 - < 10 years (0/9.8) and ≥ 10 years (4/2.9). The SMR was 1.4 (95% CI 0.4 - 3.5) for prostate cancer in the ≥ 10 year duration category. The Alexander (2001a) investigation improved upon the methods used for exposure assessment, nevertheless, some misclassification of exposure is likely. Maintenance and other mobile workers not specifically identified as definitely PFOA exposed workers may have routinely entered the areas of high exposure (drying and packaging). The extent to which this misclassification occurred and the effects on the study results is unknown.

Among the Decatur fluorochemical production workforce, there have been no prostate cancer deaths (1.0 expected) (Alexander, 2001b). In the episodes of care investigation of this same workforce with 10 or more years of experience, however, a risk ratio of 8.2 (0.8-399) was reported for prostate malignant neoplasms based on 4 episodes of care among fluorochemical workers (1.5 expected) compared to 1 episode of care among the comparison film plant workers (3.1 expected) (Olsen *et al.*, 2001b). On the other hand, there was no evidence of prostatic hypertrophy as the episodes of care risk ratio was 1.0 (95% CI 0.6-1.5) based on 24 individual episodes of care in the Decatur fluorochemical plant and 52 episodes of care in the film plant.

Conclusions

At the exposure levels encountered in either the workplace or the environment, PFOA does not appear to present a human health risk. The chemical is not genotoxic in assays measuring various endpoints and utilizing test systems ranging from bacteria to mammals. The developing fetus is not uniquely sensitive to the effects of PFOA. Indications of a fetal response are seen only under dosing/exposure conditions in which the adult animal is also responding. No evidence of structural abnormalities produced by *in utero* exposure to PFOA exists from animal tests. Clearly, the effects observed in the two-generation reproduction study (decreased pup weights, increased pup mortality, and sexual maturation delays only at the 30 mg/kg dose) did not compromise the reproductive success (i.e., mating and fertility) of PFOA-exposed rats. With

respect to the human experience there is no evidence of increases in episodes of medical care related to either developmental or reproductive health matters. In addition, evaluation of the hormonal status of 3M workers from the Cottage Grove, MN plant did not reveal any changes in sex hormones associated with PFOA exposure.

In the long-term studies with PFOA in rats, the incidence of tumors of the liver, pancreas, and testes was increased. An apparent increase in mammary fibroadenomas, seen in the PFOA-treated females, was the result of an unusually low incidence of fibroadenomas in this particular control group. The incidence of mammary tumors in all test groups was within the range expected for this strain of rat based on historical control data.

The tumors whose incidence is increased in rats treated with PFOA (liver, testes and pancreas) are frequently observed in rats treated with PPs. It is generally recognized that rats have a heightened response to peroxisome proliferators relative to other species, including man, due in part due to their higher level of expression of the nuclear receptor PPARα. Because of the increased sensitivity of rats to PPs, the human significance of these three tumor types is not clear. With respect to the liver, tumors observed in rats result from PPARα activation and are unlikely to be relevant to humans. The relevance to humans of pancreatic acinar cell tumors and Leydig cell tumors is also questionable. In addition, available data for humans who have had long-term treatment with hypolipidemic drugs (which are potent peroxisome proliferators in rats) show no increase in these three cancers associated with their long-term use.

Studies of workers, believed to be the highest exposed human population, have not shown an increased cancer risk. Mortality studies show no increase in any cancer that could be associated with PFOA exposure. In addition, the episodes-of-care study and clinical studies of workers do not reveal any indications of PFOA-related response of liver, testes, and pancreas.

In summary, it can be concluded from toxicological studies that PFOA is non-genotoxic, the fetus is not uniquely sensitive, and reproductive success is not compromised. The tumor types produced by PFOA in rats are associated with peroxisome proliferation, a response that is not readily induced in man. Thus, combined with comparatively lower exposures in humans, it is unlikely that PFOA will have an adverse impact on human health with regard to these endpoints.

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EXHIBIT C-59

SOUTH WASHINGTON COUNTY Bulletin
Your online connection to South Washington County, Minnesota

3M tells residents water OK to drink

Jon Avise

South Washington County Bulletin - 02/06/2008

3M officials last week presented three possible remedies for containing the perfluorochemical-contaminated groundwater at its Cottage Grove facility last week, taking responsibility for the required cleanup while telling residents their water is unequivocally safe to drink — even with PFCs present.

The Maplewood-based company rolled out a team of experts to present the results of 3M's environmental research at the 60-year-old Chemolite Plant in southern Cottage Grove and answer questions from residents concerned about contaminated drinking water.

Well water in nearby neighborhoods has been found to contain perfluorooctane sulfonate (PFOS), perfluorooctanoic acid (PFOA) and perfluorobutanoic acid (PFBA), chemicals manufactured by 3M until early this decade for use in a variety of non-stick or stain-resistant products.

PFBA — which exits the body more quickly than its close PFC counterparts — is also present in Cottage Grove's municipal drinking water, at levels deemed safe by state health officials.

Residents shouldn't worry, though, 3M officials said Thursday night. PFCs have not been found to have any human health effects and pose "a risk that is so low it's indistinguishable from zero," said Carol Ley, a 3M occupational health official.

But the state health department has been more cautious, continuing to release fish consumption warnings last week after further testing on metro area lakes revealed PFCs were present in many, including Cottage Grove's Ravine Lake.

Attendees were cordial to the corporate officials at the meeting, which was less tense than the informational session two nights prior with Minnesota Department of Health officials.

Despite the concerns, residents aren't angry at 3M, said Cottage Grove resident Paul Seaton, whose private well is contaminated. The company "did what was legal to do when they did it," he said of the production and disposal of the chemicals that began in 1948. "It was the best accepted practice at the time."

What locals are peeved with is a sense that 3M "is kind of avoiding taking care of the problem," Seaton said. "They have a vested interest in telling us what we want to hear," he said after the two hour-long meeting at Cottage Grove Junior High, adding, "no, I don't feel comfortable drinking that water unfiltered."

The source of Seaton and others' well contamination isn't known, 3M said Thursday, but it is likely a former 3M disposal site in Woodbury could be the source of the tainted drinking water, not the Cottage Grove facility.

That facility already has a system of deep wells installed, collecting roughly 4 million gallons of water per day from an area around the old dump in an effort to contain PFC contamination in the soil and groundwater. The millions of gallons of water captured there are transferred directly south via pipe to the Cottage Grove facility, where it is used as a coolant, then discharged legally into the Mississippi River.

The three remedial options presented for 3M's Cottage Grove site, required as part of an agreement with the Minnesota Pollution Control Agency, include similar wells in addition to varying amounts of soil extraction intended to expedite and expand the effort to remove PFCs from the site and prevent the chemicals from migrating off the company's property into local groundwater.

Speeding up that process is relative, though. Officials said they expected the remedial pumping at the Woodbury site to continue for roughly 100 years.

What are homeowners to do in the meantime?

Seaton, as well as Langdon residents Joe Murphy and Anne Redmond, said the feeling is that 3M is neglecting to take care of the residual problems from the PFCs on the company's property.

Those in the affected areas expressed concern about decreased property values due to their contaminated wells — whether or not the water is safe — and some elderly homeowners are worried they won't be able to sell their house.

"Are you going to buy a house for $200,000 and have the guy tell you there are filters in the basement because the water is contaminated?" Murphy asked.

ROWE025095

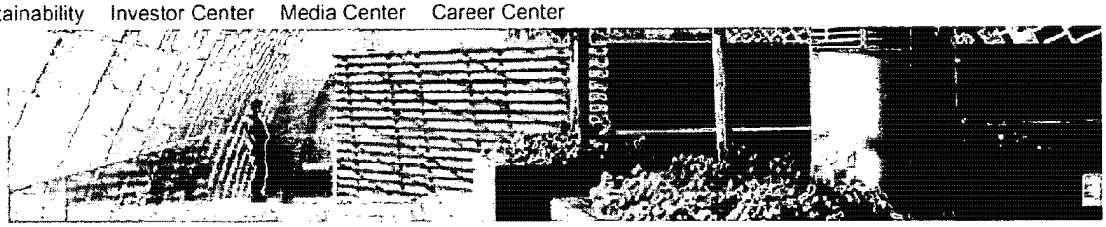
As the junior high emptied Thursday night, two long-time Cottage Grove residents commented to each other as they exited the school, even after all the questions and all the concerns, that they hadn't seen any side effects in two very familiar test subjects — themselves.

"How long have we been drinking this water? A long time," one attendee asked, adding "we're still here, aren't we?"

Jon Avise can be reached at javise@swcbulletin.com.

ROWE025096

EXHIBIT C-60



PFOA and Thyroid Changes

You may have seen or heard about a recent study about a potential relationship between PFOA and thyroid changes. PFOA is a chemical used in the manufacture of some fluoropolymers, including Teflon® non-stick coatings.* It is not used in the manufacture of stain repellents, but it can be an unintended by-product found at trace levels.

A study by Melzer, et.al., "Association Between Serum Perfluorooctanoic Acid (PFOA) and Thyroid Disease in the NHANES Study," published in Environmental Health Perspectives in January 2010, attempted to determine whether there is a potential relationship between PFOA and thyroid changes. As indicated by the authors of this study, the observed association is a correlation which may or may not be causal.

In addition, these results are not consistent with other studies, including epidemiological studies of workers. Workers in these studies had much higher levels of PFOA exposure than the general public, yet did not show any changes that would impact the thyroid.

In a community study by Emmett, et.al., published in the Aug. 2006 issue of the Journal of Occupational and Environmental Medicine, the authors found "no significant positive relationships between serum (PFOA) and thyroid-stimulating hormone." They stated, "A history of thyroid disease was quite prevalent in this population, being reported by 11% of participants, but we detected no contribution to this burden from PFOA exposure."

There continues to be a great deal of data generated through studies on PFOA and human health. We routinely review these studies, and based on the weight of evidence, we do not believe PFOA poses a health risk to our workers or to the general public.

Please visit
http://www2.dupont.com/Teflon/en_US/products/safety/index.htm
and www.pfoa.dupont.com for additional information.

ROWE050151

*Studies indicate that if any PFOA is present in cookware coatings, it is of such small amounts that the potential for any consumer exposure to PFOA is negligible. For more information, please visit the U.S. EPA web site http://www.epa.gov/oppt/pfoa.

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EXHIBIT C-61

Estimation of "Safe" Reference Level (ppb) of PFOS in Plasma

Basic Factors:

- LOAEL in Rhesus Monkeys = 0.5 mg/kg/day over 90 days.
- PFOS is cumulative, the excretion kinetic being quite low (30.2% in urine and 12.6% in feces 89 days after an i.v. dose in rats [*rats may have significantly more urinary excretion capability than humans*]).
- Absorption from the G.I. tract is almost complete (>95%) within 24 hours after administration.
- Significant enterohepatic re-circulation occurs.

Safety Factors:

- 10 for LOAEL to NOAEL
- 10 for sub-chronic to chronic
- 100 for interspecies extrapolation
- 10 for exposure of children (Food Quality Safety Act & Water Act)

note bene:
I might be inclined to keep or reduce interspecies S.F. and increase LOAEL to NOAEL and/or subchronic to chronic.

Applying 100,000-fold safety factor to sub-chonic Rhesus study gives RfD of 5 nanograms/kg/day. For a 70 kg human, this is converted to 0.35 micrograms/day.

note bene:
Please recall that FDA uses 5.0 micrograms/day from all sources as a risk level for N-Ethyl FOSE. We can compare PFOS and N-Ethyl FOSE risk values stated here on a molar basis:

- *5.0 micrograms/day N-Ethyl FOSE = 8.5 nanomoles/day*
- *0.35 micrograms/day PFOS = 0.7 nanomoles/day*

Since our basis is a 90-day study in Rhesus monkeys, what is the estimated plasma level of PFOS associated with this RfD of 0.35 micrograms per day? I can only think to do this by multiplying 0.35 micrograms per day by 90 days to get a total dose of 31.5 micrograms. Of this, let's assume 10% is in plasma (may actually be a little less). So, 3.15 micrograms in plasma gives a concentration of 1.5 ppb, assuming that the average human has 3 liters of plasma (60% of blood volume, which is 5 liters in the normal human, assuming normal hematocrit of 40%).

Therefore, I derive with **1.05 ppb** as a reference level in plasma for chronic PFOS exposure from all sources.

While this may seem extreme, it follows the approach used by federal agencies. The safety factors used could be significantly reduced with the results of additional studies.

EXHIBIT C-62

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
REGION III
1650 Arch Street
Philadelphia, Pennsylvania 19103-2029

JAN 11 2018

Mr. Andrew Hartten
Principal Project Manager-Corporate Remediation
The Chemours Company
1007 Market Street, #3094
Wilmington, DE 19899

Re: Request for sampling; GenX in water supplies

Dear Mr. Hartten:

The Environmental Protection Agency (EPA) Regions 3 and 5 have issued a series of Safe Drinking Water Act Orders in 2002, 2006 and 2009 to E.I. du Pont de Nemours and Company (DuPont) concerning the contamination of numerous public and private drinking water supplies with Perfluorooctanoic acid (PFOA), in the vicinity of the Washington Works facility located in Parkersburg, West Virginia. PFOA is a processing aid used in the manufacture of Teflon. PFOA had been released from the facility and leached into the ground water serving the water supplies in areas of West Virginia and Ohio. In February of 2015, The Chemours Company (Chemours) was formed as a wholly-owned subsidiary of DuPont. It is EPA's understanding that, at that time, Chemours took over ownership and operation of the Washington Works facility. Shortly thereafter, in July of 2015, Chemours became an independent publicly-traded company. In January of 2017, EPA, DuPont and Chemours amended a 2009 Safe Drinking Water Action Section 1431 Consent Order with DuPont to, among other things, add Chemours as a respondent and require both DuPont and Chemours to preliminarily provide temporary alternative drinking water to users of drinking water systems contaminated with PFOA concentrations exceeding 70 parts per trillion (ppt), and subsequently to treat and monitor those affected drinking water systems.

According to DuPont and Chemours, in 2013, DuPont discontinued the use and discharge of PFOA at the Washington Works facility. EPA understands that DuPont and Chemours replaced PFOA with the compound GenX, which is manufactured at the Chemours facility in Fayetteville, North Carolina. Over the past few years, the compound GenX has been identified by EPA and the North Carolina Department of Environmental Quality in the Cape Fear River, several water supplies downstream from that facility, and in groundwater wells at and around the Fayetteville facility. Chemours is currently providing bottled water to residential well owners in the vicinity of the Fayetteville facility whose drinking water samples showed levels of GenX above North Carolina's health goal of 140 ppt. EPA is concerned that drinking water wells in the vicinity of the Washington Works facility may similarly be contaminated by GenX. This concern is based in part upon the fact that

GenX has been detected in three on-site production wells and one on-site drinking water well, at the Washington Works facility.

EPA requests that Chemours sample a select group of public and private drinking water supplies for GenX in the vicinity of the Washington Works facility. The water supplies on the enclosed list were chosen by EPA based upon their historically high concentrations of PFOA. It is likely that these same wells would be impacted by GenX based upon the common methods of dispersal. Each of the selected water supplies is currently being treated with granulated activated carbon for PFOA removal. Chemours should collect GenX samples from both the raw (untreated) and the finished (treated) water in order to determine whether GenX is present, and if so, in what concentrations, as well as to determine the GAC treatment system's ability to remove GenX. Please initiate GenX testing in the next round of regularly scheduled monitoring for the identified drinking water systems, but no later than March 31, 2018. Thank you for your cooperation in this matter. If you have any questions, please contact Roger Reinhart or Jennifer Wilson at 215-814-5462 and 312-353-3115, respectively.

Sincerely,

Kate McManus, Acting Director
Water Protection Division

Enclosure

Cc: Bradley Aulick, Esq. The Chemours Company

GenX Sampling Locations near Chemours Washington Works facility – Washington, WV

Ohio Sampling Locations

- Public Water Systems
  - Belpre Public Works (PWS ID: OH8400012)
  - Little Hocking Water Association (PWS ID: OH8400212)

- Private Drinking Water Wells
  - 138 Emory Lane
  - 996 Mill Branch Road
  - 2481 State Route 124
  - 3986 Braun Road
  - 2128 State Route 339

West Virginia Sampling Locations

- Public Water Systems
  - Lubeck Public Service District (PWS ID: WV3305404)
  - Chemours Washington Works (PWS ID: WV9954007)

- Private Drinking Water Wells
  - 1672 Club Drive
  - 1652 Club Drive
  - 321 Walker Lane
  - 91 South Street
  - 1027 Low Gap River Road

EXHIBIT C-63

LITTLE HOCKING WATER ASSOCIATION, INC.
3998 Newbury Rd. • P.O. Box 188 • Little Hocking, OH 45742
(740) 989-2181 Fax (740) 989-5543
Website: www.littlehockingwater.org

GenX DETECTION NOTICE

March, 2018

[redacted]

Little Hocking Water Association is sending you this Notice to advise you that one round of water samples collected on February 19, 2018 shows the presence of an unregulated chemical known as "GenX" in Little Hocking's pretreated water at 32 parts per trillion (ppt). The same round of water testing showed the drinking water you use was non-detect for GenX. These non-detect results were taken at test locations in the Treatment Plant both between and after the Little Hocking carbon beds.

This single round of tests means there is GenX in our wellfield, but none has yet been detected in our finished water.

Chemours recently provided Little Hocking with this first round of test results. Chemours uses GenX in its manufacturing operations at the Washington Works plant in Wood County, West Virginia. The plant is immediately across the Ohio River from Little Hocking's wellfields.

GenX is the chemical that DuPont and Chemours have been using to replace C8 or PFOA. High levels of GenX have recently been found in the DuPont/Chemours plant in North Carolina that manufactures GenX. The alarming levels in North Carolina caused the US EPA to ask Chemours to test water systems in Ohio and West Virginia, where the replacement chemical is used in place of C8.

Little Hocking is taking immediate steps to verify these results and the protocols and procedures used for the sampling and analysis. Little Hocking will continue to update you on these efforts.

Background

GenX (hexafluoropropylene oxide dimer acid) is in the same class of chemicals as C8. Chemours uses GenX at the Washington Works plant in Wood County, West Virginia as an aid in the production of fluoropolymers. GenX began to draw attention from regulators when, in late 2016, testing revealed that Chemours had contaminated rivers and groundwater in North Carolina with GenX.

In January 2018, the US EPA asked DuPont/Chemours to test water supplies near Washington Works, including Little Hocking's water before and after carbon treatment. The US EPA made this request after GenX was detected in three on-site production wells and one on-site drinking water well at the Washington Works facility. The US EPA had concerns that drinking water wells in the vicinity of the Washington Works facility may similarly be contaminated by GenX.

Little Hocking promptly authorized Chemours to conduct GenX sampling on February 19, 2018 and received the results in March, 2018. GenX was present in Little Hocking's pretreated water at 32 ppt. Little Hocking's water between and after the carbon beds was non-detect. To date the federal government has not set a regulatory standard for GenX in drinking water. North Carolina, currently the only state with a regulatory level, has set a 140 ppt as the health goal for GenX in water.

Possible Public Health Risks Associated With GenX

Little Hocking believes it is important to inform you of the water consumption concerns that may be associated with exposure to GenX. GenX shares some of the same chemical properties as C8, such as toxicity to humans and animals and persistence in the environment. There is currently no research on the effects of GenX on humans, only laboratory studies on animals. This research indicates that GenX may result in liver tumors, liver disease, kidney disease, and lower birth weight in exposed animals. The Netherlands has classified GenX as a suspected human carcinogen due to these animal studies. In addition, even though GenX was not detected in our finished water in the single test taken, there are scientific concerns about the long-term effectiveness of carbon treatment at removing GenX from public water supplies.

As of this date, there is a serious question as to whether the kind of carbon filtration used at Little Hocking will effectively remove any GenX before it enters your drinking water.

Next Steps

The Board of Trustees and staff of Little Hocking, together with its consultants and lawyers, are working hard to obtain complete information related to GenX and related to the scope of GenX contamination (including the possibility of other contaminants). We are also working to verify the information that Chemours has provided, including the sampling and laboratory procedures and protocols used. To keep you apprised of the status of the issue, we will post updated information on our website at http://www.littlehockingwater.org/.

You can also contact us for additional information:

Little Hocking Water Association, Inc.
Attn: Jon T. Hanning, General Manager
3998 Newbury Road
P.O. Box 188
Little Hocking, OH 45742
(740) 989-2181

Please share this information with all other people who use this water, especially those who may not have received this notice directly (for example, people in apartments, nursing homes, schools, and businesses). You can do this by posting this notice in a public place or distributing copies by hand or mail.

Little Hocking thanks you for your patience as we continue to investigate this issue, and we regret any inconvenience these circumstances may cause.

Very Truly Yours,
Little Hocking Water

By Jon T. Hanning

Jon T. Hanning
General Manager
Little Hocking Water Association, Inc.

EXHIBIT C-64

ehp ENVIRONMENTAL HEALTH PERSPECTIVES
ehponline.org

Isomer Profiles of Perfluorochemicals in Matched Maternal, Cord and House Dust Samples: Manufacturing Sources and Transplacental Transfer

Sanjay Beesoon, Glenys M. Webster, Mahiba Shoeib, Tom Harner, Jonathan P. Benskin, Jonathan W. Martin

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Online 14 July 2011

NIEHS
National Institute of Environmental Health Sciences

National Institutes of Health
U.S. Department of Health and Human Services

Isomer Profiles of Perfluorochemicals in Matched Maternal, Cord and House Dust Samples: Manufacturing Sources and Transplacental Transfer

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Running Title: PFC Isomer Profiles in Blood and Dust.

Key words: Isomers, Perfluorochemicals, PFOA, PFOS, Transplacental transfer,

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Competing Interests: None

Abbreviations and definitions:

ECF	- Electrochemical fluorination
PFBS	- Perfluorobutane sulfonate
PFC	- Perfluorochemical
PFDA	- Perfluorodecanoate
PFDoA	- Pefluorododecanoate
PFDS	- Perfluorodecane sulfonate
PFHxA	- Perfluorohexanoate
PFHxS	- Perfluorohexane sulfonate
PFNA	- Perfluorononanoate
PFOA	- Perfluorooctanoate
PFOS	- Perfluorooctane sulfonate
PFTA	- Perfluorotetradecanoate
PFUnA	- Perfluoroundecanoate

Abstract

BACKGROUND: Perfluorochemicals are detectable in the general population and in the human environment, including house dust. Sources are not well-characterized, but isomer patterns should enable differentiation of historical and contemporary manufacturing sources. Isomer-specific maternal-fetal transfer of perfluorochemicals has not been examined despite known rodent developmental toxicity of perfluorooctanesulfonate (PFOS) and perfluorooctanoate (PFOA).

OBJECTIVES: To elucidate relative contributions of electrochemical (phased out in 2001) and telomer (contemporary) perfluorochemicals in dust, and to measure how transplacental transfer efficiency (TTE, based on a comparison of maternal and cord sera concentrations) is affected by perfluorinated chain-length and isomer branching pattern.

METHODS: Matching samples of house dust (n=18), maternal sera (n=20) and umbilical cord-sera (n=20) were analyzed by isomer specific HPLC-MS/MS.

RESULTS: PFOA isomer signatures revealed that telomer sources accounted for 0 to 95% of total PFOA in house dust (median = 31 %). This may partly explain why serum PFOA concentrations are not declining in some countries despite the phase-out of electrochemical PFOA. TTE data indicate that total branched isomers crossed the placenta more efficiently than linear isomers for both PFOS ($p<0.01$) and PFOA ($p=0.02$), and that placental transfer of branched isomers of PFOS increased as the branching point moved closer to the sulfonate (SO_3^-) end of the molecule.

CONCLUSIONS: Results suggest that humans are exposed to telomer PFOA, but larger studies that also account for dietary sources should be conducted. The exposure profile of PFOS and PFOA isomers can differ between the mother and fetus, an important consideration for perinatal epidemiology studies of perfluorochemicals.

Introduction

The most prominent perfluorochemicals (PFCs) in human samples are perfluorooctane sulfonate (PFOS), perfluorooctanoate (PFOA), and perfluorohexane sulfonate (PFHxS), yet the sources and pathways of human exposure to these, and other PFCs, are not well-characterized. Perfluorinated acids are ubiquitous in the global environment, owing to their long history of manufacture and resistance to biological and environmental degradation pathways. Specifically for PFOA, the manufacturing sources responsible for its presence in various environments are not understood, and future human exposure is therefore difficult to predict. There are 2 main manufacturing methods leading to PFOS and PFOA, electrochemical fluorination (ECF) and telomerization. The 3M Co. manufactured the bulk of PFOS (and higher molecular weight precursor materials), PFHxS, and PFOA by ECF until 2001, at which time they voluntarily phased-out these chemistries. Nonetheless, PFOS and its precursors continue to be manufactured by other companies in Asia (Martin et al. 2010). Telomerization continues to be used to manufacture PFOA. ECF and telomerized PFOA can be readily distinguished analytically because ECF PFOA consists of a mix of linear and branched isomers (Loveless et al. 2006, Reagen et al. 2007), while telomerized PFOA is almost exclusively the linear isomer (Kissa 1994).

If humans are predominantly exposed to ECF sources of PFOS and PFOA, serum concentrations should be decreasing due to their phase-out. In fact, when the 3M Co. stopped manufacturing PFOS and PFOA by its ECF technique, blood levels of PFOS declined steadily in Americans. However, for PFOA the initial rate of decline was much less than anticipated (Olsen et al. 2008), and the most recent data from the National Center of Health Statistics of the US Center for Disease Control (Kato et al. 2011) indicate that serum PFOA did not decline between

2003/04 and 2007/08, and may be increasing (Supplemental Material-Figure 1). This suggests that exposure to recently-produced telomer sources of PFOA might be important, but the relative importance of ECF- and telomer-derived PFC exposures through different exposure pathways (diet, dust, water, air, etc.) is unknown. Nonetheless, the potential for telomer PFOA exposure is recognized, and in 2006 a global stewardship program was implemented to reduce emissions of this chemical (U.S. EPA 2006).

For many environmental chemicals, house dust can be a major source of exposure (Butte et al. 2002), particularly for children (US EPA 2008). For PFOS and PFOA, food is a major source of exposure, but house dust can also be important under scenarios of high dust-ingestion (Tittlemeir et al. 2007, Bjorklund et al. 2009, Goosey and Harrad 2011, Shoeib et al. 2011, Haug et al. 2011). Thus, it is important from a risk mitigation perspective to understand whether PFCs in house dust are from current or historical manufacturing sources. PFOS and PFOA have been measured in dust previously (Kato et al. 2009, Kubwabo et al. 2005), but isomer specific PFC analytical methods (Benskin et al. 2007; Langlois and Oehme 2006) have not been used to determine the manufacturing origins of PFOA and other PFCs in house dust.

Loveless et al. (2006) demonstrated that linear ammonium PFOA was generally more toxic than branched PFOA, but the isomer specific toxicity of PFCs have not been examined because of the lack of available standards. Studies in rats and zebrafish show that PFOA and PFOS are developmental toxicants (Lau et al. 2004), and many human epidemiology studies are now emerging on the potential perinatal effects of PFCs. For example, some epidemiology studies have shown inverse associations between PFOA exposure and birth weight (Apelberg et al. 2007; Fei et al. 2008), while others did not find any association (Monroy et al. 2008; Nolan et al. 2009). Furthermore, other adverse human health effects associated with PFCs are being

detected in both background (Nelson et al. 2010) and highly exposed populations (Steenland et al. 2010). From a public health perspective, and recognizing that many PFCs occur as multiple isomers of unknown relative toxicity, it may be important to characterize the exact nature of PFC exposure to humans, including for the mother and the fetus.

Understanding the maternal-fetal transmission of PFCs is necessary to clearly understand the risks and mechanisms of human developmental toxicity. Of studies that have reported the maternal-fetal transfer of PFCs, Hanssen et al. (2010) was the only study to examine branched isomers separately from linear isomers. However, individual branched isomers were not examined separately (i.e. total branched PFOS was compared to linear PFOS). Although we are beginning to understand the pharmacokinetic properties of specific branched PFCs in animal models (De Silva et al. 2009), no study has yet investigated isomer-specific PFC pharmacokinetics in humans. In an attempt to understand the transplacental transfer of PFCs (mainly PFOS and PFOA), multiple studies have tested maternal and umbilical cord blood samples from different populations (Needham et al. 2011, Kim et al. 2011, Fromme et al. 2010, Hanssen et al. 2010, Monroy et al. 2008, Midasch et al. 2008, Fei et al. 2007, Inoue et al. 2004). One consistent finding in all these studies is that cord serum has lower total PFOA and lower total PFOS than maternal serum; however the isomer-specific transplacental transfer of the various branched isomers has not been examined despite evidence that the placental transfer of total branched PFOS isomers is greater than for linear PFOS (Hanssen et al. 2010).

In the current work we collected dust from the homes of twenty pregnant women who also donated a blood sample at 15 weeks of gestation and a cord blood sample at delivery. We measured PFC concentrations and isomer profiles in all samples in an effort to identify sources of PFCs in house dust, and to examine the isomer specific transfer of PFCs across the placenta.

Materials and Methods

Nomenclature and Acronyms. For structural isomers, we use the nomenclature defined by Benskin et al. (2007). Using PFOS as an example, the following annotations are used to represent the structure of each isomer based on relative position of perfluoromethyl substitution: linear perfluorooctane sulfonate (*n*-PFOS), perfluoroisopropyl (*iso*-PFOS), 5-perfluoromethyl (5*m*-PFOS), 4-perfluoromethyl (4*m*-PFOS), 3-perfluoromethyl (3*m*-PFOS), 1-perfluoromethyl (1*m*-PFOS), tert-perfluorobutyl (*tb*-PFOS) and sum of all dimethyl isomers (Σm_2-PFOS). With the exception of *n*-PFOS, all of the above mentioned isomers are branched isomers. (For structures see Supplemental Material, Figure 2).

PFC Chemical Standards. The 3M Co. donated ECF PFOS (30 % branched and 70 % linear, by ^{19}F NMR) and PFOA (22 % branched and 78 % linear, by ^{19}F NMR) standards (Reagen et al. 2007). All other PFC standards, including PFOS and PFOA isomer standards, and linear mass-labeled internal standards for PFBA, perfluorohexanoate (PFHxA), PFHxS, PFOA, perfluorononanoate (PFNA), PFOS, perfluorodecanoate (PFDA), perfluoroundecanoate (PFUnA) and perfluorododecanoate (PFDoA) (MPFAC-MXA), were obtained from Wellington Laboratories (Guelph, ON, Canada).

Blood Collection. Samples (n=20) analyzed in this study are a subset of participants recruited in 2007-2008 to a Canadian (Vancouver, BC) cohort study named Chemicals, Health and Pregnancy (CHirP) (Webster et al. 2011). All participants provided informed consent. Laboratory personnel collected blood samples from pregnant volunteers in Vancouver, BC, at 15 weeks of gestation, and samples of cord blood (n=20) were collected at delivery. After serum

separation, all samples were stored at -80 °C. Ethical clearance was obtained from the Research Ethics Boards of the University of British Columbia, the University of Alberta, Health Canada, and the 3 participating hospitals.

Serum Preparation. The method of Kuklenyik et al. (2004) was adapted for the extraction of PFCs from serum using a Rapid Trace system (Caliper Life Sciences, Hopkinton, MA). The solid phase extraction cartridge (Oasis-HLB, Waters, 60 mg/3mL) was conditioned with 2 mL of methanol followed by 2 mL of 0.1 M formic acid. Serum was prepared for extraction by mixing 3 mL of 0.1 M formic acid with 1.0 mL of serum. Following the addition of mass labelled internal standards (10 ng of each), the mixture was vortexed and sonicated for 20 min. Prepared serum was added to the column and washed successively with 3 mL of 0.1 M formic acid, 6 mL of 50 % 0.1M formic acid/50 % methanol, and 1 mL of 1 % ammonium hydroxide. The cartridge was drained by vacuum and PFCs were eluted with 1.0 mL of 1 % ammonium hydroxide in acetonitrile. The eluate was concentrated to 100 µL followed by the addition of 200 µL of 90 % 20 mM acetic acid in 10 % methanol. Method blanks containing calf serum, a calf serum sample spiked at 0.5 ng/mL of each PFC, and a human serum sample spiked at 10 ng/mL were analyzed with the real samples.

Dust Collection and Extraction. Dust samples were also a subset of those collected for the larger CHirP study (Shoeib et al. 2011). At 20-24 weeks of gestation, participants donated a used vacuum cleaner bag from their vacuum cleaner, or we took grab samples from the participants' bagless vacuum cleaners (n=18). Samples were stored at -20 °C, and before analysis a portion of each dust sample was sieved using a stainless steel sieve (mesh size 150 µm, VWR International,

Montreal, Quebec). Sieved dust (0.1 g) was spiked with 3.3 ng of mass labeled internal standards (MPFAC-MXA), and 4 mL of methanol was added and vortexed for 5 min, sonicated for 1 hr, and centrifuged at 3400 rpm for 10 min. A 2 mL aliquot was reduced by evaporation to 100 µL, and 200 µL of 20 mM acetic acid with 10 % methanol was added before HPLC-MS/MS.

Total PFC Analysis. For total PFC concentrations, separation was by HPLC on a 150 mm Synergi™ Hydro-RP C-18 column (Phenomenex, Torrance, California). Gradient elution at 600 µL/min used A (20 mM ammonium acetate (pH 4) in water) and B (methanol) mobile phases. Initial conditions were 60 % A for 1 min, ramped to 20 % A by 3 min followed by a 5 min hold and a subsequent increase to 100 % B by 8.5 min, held until 14 min at which time initial conditions were reestablished. Tandem mass spectrometry (MS/MS) data were collected on an Applied Biosystems API 3000 (Carlsbad, California), using electrospray ionization in negative ion mode. For mean recoveries of total PFCs in serum and dust, see Supplemental Material, Table 1.

Isomer Specific PFC Analysis. The isomer-specific HPLC-MS/MS method was adapted from Benskin et al. (2007). Briefly, 3 µL of the same extracts analyzed for total PFCs were injected onto a FluoroSep RP Octyl column (ES Industries, West Berlin, NJ). Flow rate was 200 µL/min, and starting conditions were 60 % A (water adjusted to pH 4.0 with ammonium formate) and 40 % B (methanol). Initial conditions were held for 0.3 min, ramped to 64 % B by 1.9 min, increased to 66 % B by 5.9 min, 70 % B by 7.9 min, 78 % B by 40 min, 88 % B by 42 min and finally to 100 % B by 45 min and held until 60 min. Mass spectral data were collected using a 5000Q mass spectrometer (MDS Sciex, Concord, ON, Canada) equipped with an electrospray

interface operating in negative ion mode. Chromatograms were recorded by multiple reaction monitoring (MRM) with 3 to 13 transitions per analyte.

Quality Control. Triplicate recovery experiments were performed at 2 concentrations of native linear standards spiked to calf serum or dust (see Supplemental Material, Table 1). There are no mass-labeled internal standards for branched PFOS or PFOA isomers, thus a standard addition experiment was done in dust to rule out possible matrix effects on the measured isomer profiles. Additionally, a vacuuming experiment was done to check if off-gassing during vacuuming may bias the dust isomer profile. The results of these two experiments clearly showed that matrix effects and off-gassing during vacuuming were not a problem. The % recovery during serum extraction was similar for all PFOS and PFOA isomers, such that the extraction step had no effect on the resulting isomer profiles (Benskin et al. 2007).

Results and Discussion

Total PFC Concentrations in House Dust. All total PFCs, except for PFDS, were log-normally distributed (Shapiro-Wilk test, see Supplemental Material, Table 2 for distributions). The three major PFCs in all dust samples (n=18) were PFOA, PFOS, and PFHxA, with similar median values of 38, 37 and 35 ng/g, respectively. However, PFHxS exceeded PFOS in 4 samples. This pattern, whereby PFOA, PFOS, and PFHxA were the dominant PFCs, is not dissimilar from Strynar and Lindstrom (2008) who monitored US house dust collected in 2001/2002 and found median PFOA, PFOS and PFHxA concentrations of 142, 201, and 54.2 ng/g, respectively. The higher concentrations of PFOS and PFOA observed by Strynar and Lindstrom (2008) are understandable given that these dust samples were collected years earlier than in the current

study, around the time of the phase-out of ECF C8 chemistries, though sampling strategy and geography may also have contributed to differences.

PFC Isomer Profiles in House Dust. For PFOS, six major branched isomers were detected in dust: 1*m*-, 3*m*-, 4*m*-, 5*m*-, *iso*-, and Σm_2-PFOS. All the dust samples had PFOS isomer profiles that were very similar to the 3M Co. ECF standard of PFOS, with a mean branched isomer content (±SD) of 30±2.7 %, and relatively low variability among individual branched isomers in various samples (Supplemental Material Figure 3A and Table 3). This was not surprising given that the 3M Co. produced the bulk of PFOS (Paul et al. 2009), and that the historical batch to batch variation of branched isomer content was small: 30±0.8 % branched PFOS in eighteen lots over 20 years (Reagen et al. 2007).

Unlike PFOS, PFOA isomer profiles in dust were often substantially different from the 3M ECF PFOA standard. Although the relative profile among individual branched PFOA isomers was consistent among dust samples (Supplemental Material Figure 3B and Table 3), there was an excess signal of linear PFOA in many of the samples compared to the 3M Co. ECF standard. Like 3M Co. PFOS, batches of 3M Co. PFOA also had a consistent isomer composition: 22±1.2 % branched isomers in 18 lots over 20 years (Reagen et al. 2007). Therefore, these observations suggest that a significant proportion of PFOA in these house dust samples came from a manufacturing source that used telomerization instead of ECF. The "% telomer" PFOA in each dust sample was calculated from the excess signal of linear isomer in samples (*m/z* 413/369 transition), compared to 3M Co. ECF PFOA. The % telomer ranged from 0 to 95% with a median of 31% among all samples (Figure 1). The presence of telomer PFOA in the human household environment may partly explain why total PFOA in serum has declined so slowly

following the phase out of ECF perfluorooctyl chemistries by 3M Co. in the US (Olsen et al. 2008, Supplemental Material Figure 1). However, telomer PFOA also may be present in food, and telomer PFOA-precursors used in food packaging may be absorbed and biotransformed to PFOA after ingestion (D'eon and Mabury 2011).

Isomer-specific chromatograms of house dust clearly indicated that other perfluorocarboxylates (i.e., PFCs with $-CO_2^-$ as a functional group) had only minor branched isomer content (data not shown). Authentic standards were not available for confirmation, thus we identified peaks as branched isomers only when 2 characteristic MS/MS transitions responded at the same retention time. Most perfluorocarboxylates other than PFOA appeared exclusively linear, but in a minority of dust samples PFNA had up to 4 minor branched isomers, while PFHxA, PFDA, PFUnA and PFDoA each had up to 2 minor branched isomers. The manufacturing sources of these particular branched perfluorocarboxylates cannot be confirmed due to limited information on their manufacturing sources and a lack of reference materials, but they may be residuals from ECF manufacturing of PFOS and PFOA. Perfluorosulfonates, such as PFDS, perfluoroheptane sulfonate (PFHpS), and PFHxS, are generally assumed to have been produced exclusively by ECF, and these all had major branched isomer content based on peak areas (data not shown). However, because reference materials were not available, we could not examine how closely they resembled authentic ECF manufacturing sources.

Total PFCs in Maternal and Cord Sera. Concentrations of total PFOS (n=20), PFOA (n=20), PFNA (n=20), PFDA (n=16) and PFHxS (n=8) in maternal serum were always significantly higher ($p<0.01$) than in cord serum, consistent with previous maternal-fetal transfer studies of PFOS and PFOA (Table 1). The major total PFCs in maternal and cord sera were PFOS, PFOA, PFHxS and PFNA, similar to previous findings (Inoue et al. 2004; Midasch et al. 2007; Monroy

et al. 2008). In the current study, the mean concentrations of PFOS, PFOA, PFHxS, PFNA and PFDA in maternal serum (and cord serum) were 5.5 (1.8), 1.8 (1.1), 1.7 (0.7), 0.9 (0.4) and 0.4 (0.1) ng/mL respectively. PFUnA, PFDoA and perfluorotetradecanoate (PFTA) were detected (detection limit 0.1 ng/mL) in 6, 2, and 3 maternal samples, respectively, but were not detected in any cord samples.

Transplacental transfer efficiencies (TTEs) were estimated by dividing the PFC concentrations in cord serum at delivery by maternal serum concentration at 15 weeks gestation for each mother-cord pair (Table 2). Mean TTEs were always below 1.0, indicating lower concentrations in the cord serum than maternal serum (all $p<0.01$). Overall, the PFOS and PFOA TTEs were within the range reported in the literature. However, it is likely that our TTEs slightly underestimate actual TTE values because they do not reflect hematologic changes that occur later in pregnancy, including expansion of total plasma volume (Whittaker et al. 1996). Such an effect was shown by Monroy et al. (2008) who reported lower serum PFOS and PFOA levels in maternal serum samples collected at delivery versus 24^{th}-28^{th} weeks of gestation, and by Fei et al. (2007) who reported higher cord-maternal ratios based on maternal serum samples collected during the 2^{nd} trimester than the 1^{st} trimester (Table 1). We used the above data from Monroy et al. (2008) (see Table 1, footnote e) to estimate time-of-delivery maternal serum concentrations, based on our 15-week data, but this had little effect on the resulting TTE, and both adjusted and unadjusted TTE values were within the range of TTEs reported previously (Table 1).

A comparison of TTE among the three major perfluoroalkyl carboxylates (PFOA, PFNA, and PFDA) suggests that the longer-chain carboxylates were more efficiently blocked by the placental barrier (Figure 2A), consistent with the results of Kim et al. (2011). The same trend was also evident for the two major perfluorosulfonates (PFHxS and PFOS, Figure 2B). Overall,

shorter-chained PFCs crossed the placenta more efficiently than longer chain PFCs, consistent with the findings of Needham et al. (2011).

PFC Isomer Profiles in Maternal and Cord Sera. The % branched content of total PFOS was consistently and significantly higher in cord serum than in corresponding maternal serum and dust samples (Figure 3). Branched PFOS isomers contributed 27- 44 % (median 36 %) of total PFOS in maternal serum, and from 36-54 % (median 46 %) in cord serum. A paired t-test indicated statistically greater proportions of branched PFOS in the cord serum ($p<0.01$). Overall, all branched PFOS isomers were transferred more efficiently (median TTEs of the different branched isomers = 0.34 - 0.88) than the linear isomer (median TTE = 0.30) (Table 2). This is similar to Hanssen et al. (2010), wherein a statistically greater relative abundance of linear PFOS was reported in maternal serum than cord serum, relative to total branched PFOS isomers ($p<0.05$ by Wilcoxon's signed rank).

Unlike in Hanssen et al. (2010), where total branched PFOS isomers were quantified together, we analyzed individual branched isomers, and results suggest a structure-activity relationship for TTE. Specifically, among the perfluoromethyl PFOS branched isomers, TTE increased as the branching point moved closer to the sulfonate moiety: $1m > 3m > 4m \approx 5m > iso$ (Figure 2C). In fact, for $1m$-, $3m$-, and particularly $\sum m_2$-PFOS, the concentrations were sometimes higher in cord serum than in corresponding maternal serum (resulting in maximum TTE values > 1.0), which was never the case for total PFOS or linear PFOS (Table 2).

Branched PFOA isomers contributed 0.43-4.3 % (mean 1.9 %) of total PFOA in maternal serum, and from 0.71-5.7 % (mean 2.2 %) in cord serum. Such highly linear isomer profiles of PFOA in human serum have previously been reported (De Silva and Mabury 2006), yet it is

important to note that these cannot be used to quantitatively assess exposure sources (i.e. telomer versus electrochemical) because in animal models the branched isomers of PFOA are accumulated to a lesser extent than linear PFOA (De Silva et al. 2009). There was no structure-activity evident for PFOA isomers (Figure 2D), but a paired t-test indicated significantly higher total branched PFOA isomers in cord serum than maternal serum ($p=0.02$). In some cases, the concentrations of 5*m*-, 4*m*-, and 3*m*-PFOA were higher in the cord serum than in corresponding maternal serum (resulting in maximum TTE > 1.0), which was never the case for total PFOA or linear PFOA (Table 2).

Passive diffusion is often the mechanism by which chemicals cross the placental barrier (Syme et al. 2004), thus the TTE of hydrophilic compounds is generally lower than for hydrophobic compounds (Van der Aa et al. 1998). Based on earlier elution in reversed-phase chromatography, branched PFOS isomers are anticipated to be more hydrophilic than linear PFOS, and short-chain carboxylates (e.g. PFOA) should be more hydrophilic than longer-chain carboxylates (e.g. PFNA and PFDA), thus the current results are unexpected. However, perfluorinated acids are highly protein bound in serum (Jones et al. 2003), and the dynamics of protein binding are likely to influence TTE. For example, if the binding affinity of linear PFOS to maternal serum protein is higher than for branched PFOS isomers, there would be a higher free fraction of branched PFOS available to cross the placenta.

For the major PFCs in maternal serum we examined whether the branched isomer content was correlated to the branched isomer content in the corresponding house dust sample. While we did not observe a significant correlation between serum and dust branched isomer content for PFOS (Spearman Correlation Coefficient=-0.10, $p=0.35$) or PFHxS (Spearman Correlation Coefficient=-0.11, $p=0.33$), we found a borderline significant correlation for PFOA (Spearman

Correlation Coefficient=0.35, p=0.08). However, we cannot confirm that dust was a source of branched PFOA isomers in these women given the small sample size (n=20) and the potential contribution of other sources of exposure, including diet, water, and air (Haug et al. 2011).

In contrast with expectations, we observed a higher mean percentage of branched PFOS isomers in maternal serum [36 %, 95% confidence interval (CI) 33.6 to 38.2 %] than in historic 3M Co. ECF PFOS (30 % CI 29.3 to 30.7 %) (Reagen et al. 2007) or house dust samples (30% CI 28.6 to 31.3 %). A paired t-test showed significantly higher branched PFOS content in maternal serum than in house-dust (6% higher, 95 % CI 3.1 % - 8.9 %, p <0.001). Studies in rodents show that branched PFOS isomers are no more bioaccumulative than linear PFOS (De Silva et al. 2009), thus it would seem pharmacokinetically impossible to accumulate >30 % branched PFOS isomers if the only source of exposure was ECF PFOS. Nonetheless, Karrman et al. (2007) and Hanssen et al. (2010) also reported high proportions of branched PFOS isomers in human serum. While it is possible that PFOS isomer pharmacokinetics in humans are opposite to those in rats (De Silva et al. 2009), or that some humans are exposed to an unusually high branched PFOS source in the diet, an alternative explanation is that a significant proportion of the PFOS body burden comes from metabolism of PFOS-precursors. Benskin et al. (2009) demonstrated that branched isomers of a PFOS-precursor could be biotransformed at greater rates than the corresponding linear precursor and Haug et al. (2011) found a significant association between PFOS-precursors in air and increasing branched PFOS content of serum. In the current samples it was evident that maternal and cord serum PFOS concentrations were higher (p<0.01 for maternal serum, p=0.01 for cord serum) when N-methyl perfluorooctanesulfonamidoacetate (a PFOS-precursor) was detected in the same sample, but no

significant association was found between total dust PFOS-precursors and the branched PFOS content of serum (p=0.47).

Study Limitations. One limitation of this study is the relatively small sample size. Larger studies are recommended to elucidate the relative importance of ECF- and telomer- derived sources of PFCs to humans in other areas. The current study was not designed to test whether PFC signatures in dust were responsible for PFC signatures in maternal or cord serum; rather it was an exploratory investigation of the variability of isomer profiles in dust to elucidate manufacturing sources, and of the variation of isomer profiles between maternal and cord samples in order to investigate if branched isomers crossed the placenta to different extents.

A second limitation was that the time of sampling of pregnant women (15 wks) was relatively early in the pregnancy, and it is not clear if the isomer profile may have been different at time of delivery. For two women in our study we also analyzed 18 week serum samples, and total PFOS and individual PFOS isomers were not substantially different over these 3 weeks (data not shown). Although this is a narrow window of time, it is not an insignificant period because hematologic indices change significantly beginning as early as the 7^{th} week of pregnancy, including expansion of total blood plasma volume by 16 % between 12 and 20 weeks (Whittaker et al. 1996).

Conclusion

Both ECF and telomer manufacturing sources contributed to household dust PFOA concentrations in this exploratory study. Some homes with the highest PFOA dust concentrations had a near exclusive telomer PFOA signal, and such results may help explain why PFOA

continues to be a major contaminant of human serum despite the ECF PFOA phase-out. Larger-scale studies that examine manufacturing sources while simultaneously accounting for dietary pathways would be beneficial. It is recognized that such investigations are technically challenging because isomer profiles in biological samples (i.e. food) may bias source apportionment due to differential uptake of the various isomers. The TTE of PFCs was inversely related to chain-length, and TTEs suggest that most branched PFOA and PFOS isomers crossed the placenta to a greater extent than the corresponding linear isomer. In some cases, minor PFOA and PFOS branched isomers were more concentrated in cord serum than maternal serum, indicating that isomer-specific analysis should be performed in future studies of PFCs and birth outcomes.

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Table 1. Summary of existing studies on maternal-fetal transfer of total PFOA and total PFOS.

Study	Sampling year	Location	Sample Size	Mean Ratio of Cord to Maternal Serum Concentration (Corr. Coefficient)	
				PFOA	PFOS
Needham et al. (2011)	2000	Faroe Islands	12	0.72 (0.91)[a]	0.34 (0.82)[a]
Kim et al. (2011)	2007	Korea	20	0.69 (0.88)[a]	0.36 (0.50)[b]
Fromme et al. (2010)	2007-2009	Germany	27	0.70 (0.94)[b]	0.30 (0.89)[b]
Hanssen et al. (2010)	2005-2006	South Africa	58	0.71 (0.67)[b]	0.45 (0.88)[b]
Monroy et al. (2008)	2004-2005	Canada	101	0.81 (0.88)[a]	0.45 (0.83)[a]
Midasch et al. (2008)	2003	Germany	11	1.26 (0.72)[b]	0.60 (0.42)[b]
Fei et al. (2007)	1996-2002	Denmark	50[c]	0.55	0.29
			50[d]	0.68 (0.84)[a]	0.34 (0.72)[a]
Inoue et al. (2004)	2003	Japan	15		0.32 (0.88)[a]
Current Study	2007	Canada	20[d]	0.61 (0.63)[b]	0.33 (0.81)[b]
			20[e]	0.71 (0.76)[b]	0.36 (0.81)[b]

[a] Pearson Correlation, [b] Spearman Rank Correlation, [c] maternal serum was sampled in the 1st trimester, [d] maternal serum was sampled in the 2nd trimester, [e] TTE adjusted from 15 weeks to time of delivery (approximately 40 wks) using data of Monroy et al. (2008) whereby PFOS declined 10 % and PFOA declined 12 % between the 24th-28th week and delivery.

Table 2. Transplacental transfer efficiency (TTE) calculated by the ratio of cord: maternal serum concentrations. Values less than 1 indicate higher concentrations in maternal serum, whereas values greater than 1.0 indicate higher concentrations in cord serum.

Value	Total, linear and branched PFOS								Total, linear and branched PFOA							Other PFCs		
	Total PFOS	n-PFOS	iso PFOS	5m-PFOS	4m-PFOS	3m-PFOS	1m-PFOS	$\sum m_2$-PFOS	Total PFOA	n-PFOA	iso-PFOA	5m-PFOA	4m-PFOA	3m-PFOA	tb-PFOA	Total PFNA	Total PFDA	Total PFHxS
Arithmetic Mean	0.33	0.33	0.36	0.53	0.55	0.67	0.87	0.84	0.61	0.62	0.84	0.86	0.64	0.76	0.25	0.41	0.34	0.41
Median	0.31	0.30	0.34	0.52	0.52	0.68	0.88	0.78	0.63	0.61	0.67	0.54	0.68	0.68	0.25	0.38	0.23	0.38
Standard Deviation	0.09	0.12	0.14	0.18	0.19	0.23	0.23	0.37	0.17	0.20	0.58	0.99	0.34	0.59	0.32	0.17	0.25	0.12
Minimum	0.20	0.10	0.09	0.25	0.20	0.21	0.36	0.22	0.32	0.26	0.16	0.09	0.09	0.07	0.02	0.13	0.00	0.29
Maximum	0.53	0.58	0.60	0.93	0.85	1.12	1.24	1.72	0.96	1.00	2.56	2.26	1.29	2.74	0.48	0.78	1.10	0.56
N [a]	20	20	20	20	20	20	20	20	20	20	20	4	19	18	2	20	16	8

[a] number of maternal-cord pairs that were available for calculating TTE. When concentrations were non-detect in maternal or cord samples, that pair was excluded in the analysis. Mean TTEs were always below 1.0, indicating lower concentrations in the cord serum than maternal serum (all $p<0.01$). Linear PFOS and PFOA are denoted as n-PFOS and n-PFOA respectively.

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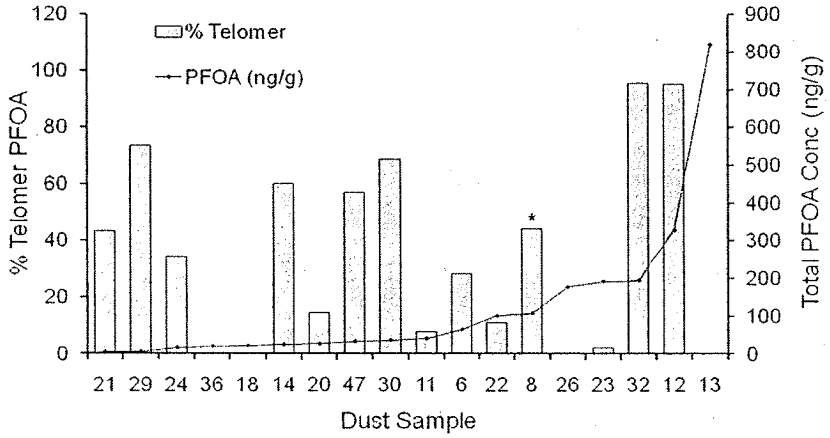
Figure Legends

Figure 1. Percent telomer PFOA (grey bars) and total PFOA concentration (ng/g, black line) in house dust samples. Samples are shown from lowest to highest total PFOA concentration, from left to right. * indicates a sample collected by mechanical sweeper, instead of vacuum.

Figure 2. TTE distributions for (A) different chain-length perfluorocarboxylates, (B) different chain-length perfluorosulfonates, (C) linear and branched PFOS isomers, and (D) linear and branched PFOA isomers. The upper and lower bounds of the boxes indicate the 75^{th} and 25^{th} percentiles, respectively, and the horizontal lines within the boxes indicate median values. The upper and lower limits of the whiskers indicate minimum and maximum values, respectively, and points above or below the whiskers indicate outlier values.

Figure 3. Percent branched PFOS isomers (Σbranched/(Σbranched+linear)) in 20 matched samples of maternal serum (15 weeks gestation) and cord serum at delivery. Samples are arranged, from left to right, by increasing branched PFOS isomer content of the maternal sample



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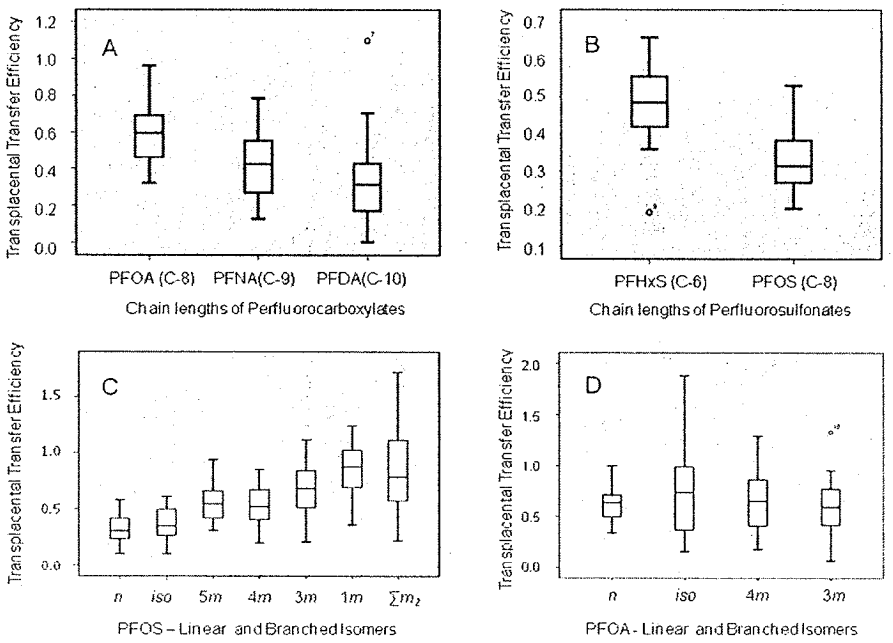

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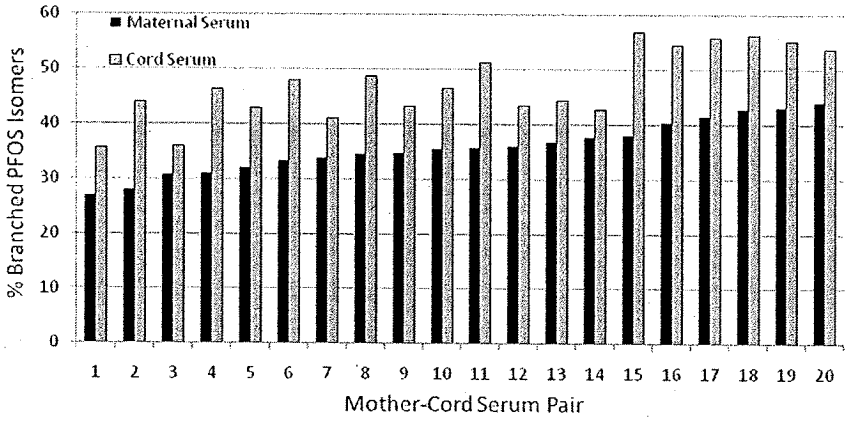

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254x190mm (96 x 96 DPI)

SUPPLEMENTAL MATERIAL

Isomer Profiles of Perfluorochemicals in Matched Maternal, Cord and House Dust Samples: Manufacturing Sources and Transplacental Transfer

Sanjay Beesoon,[1] Glenys M. Webster,[2] Mahiba Shoeib,[3] Tom Harner,[3] Jonathan P. Benskin,[1] and Jonathan W. Martin[1]

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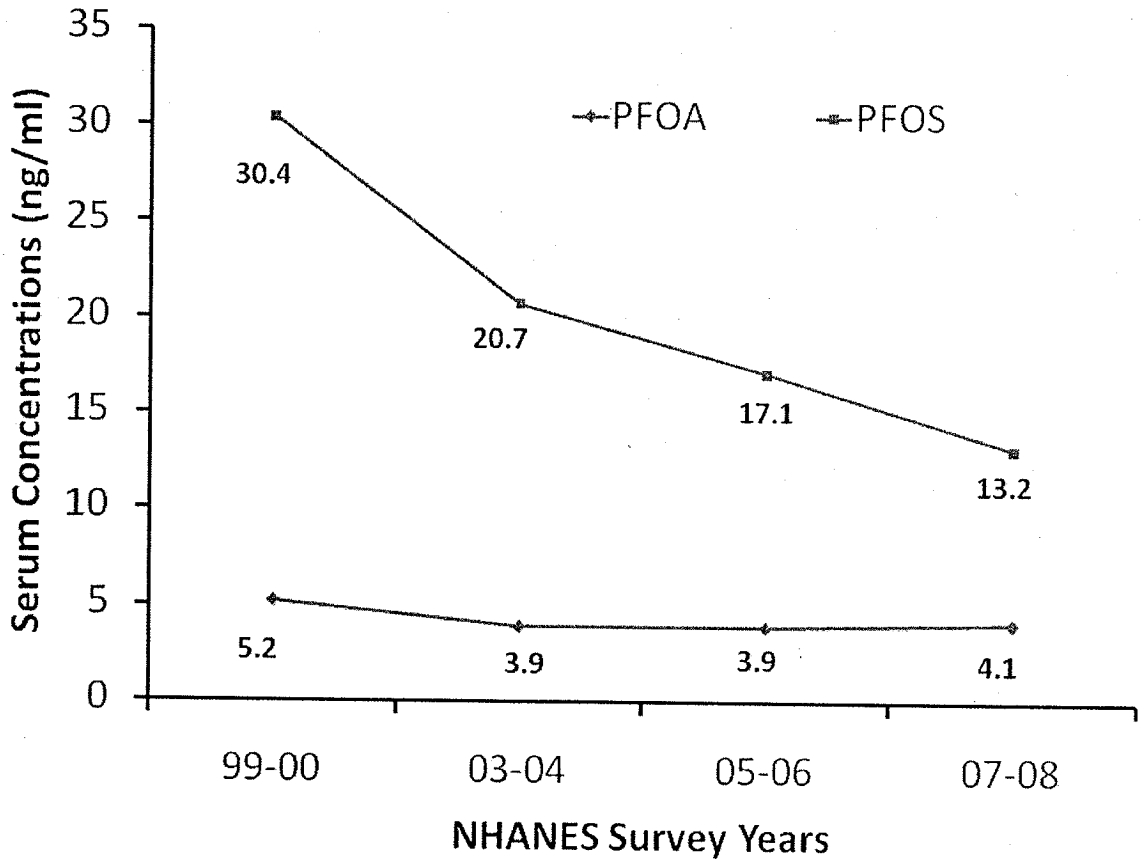
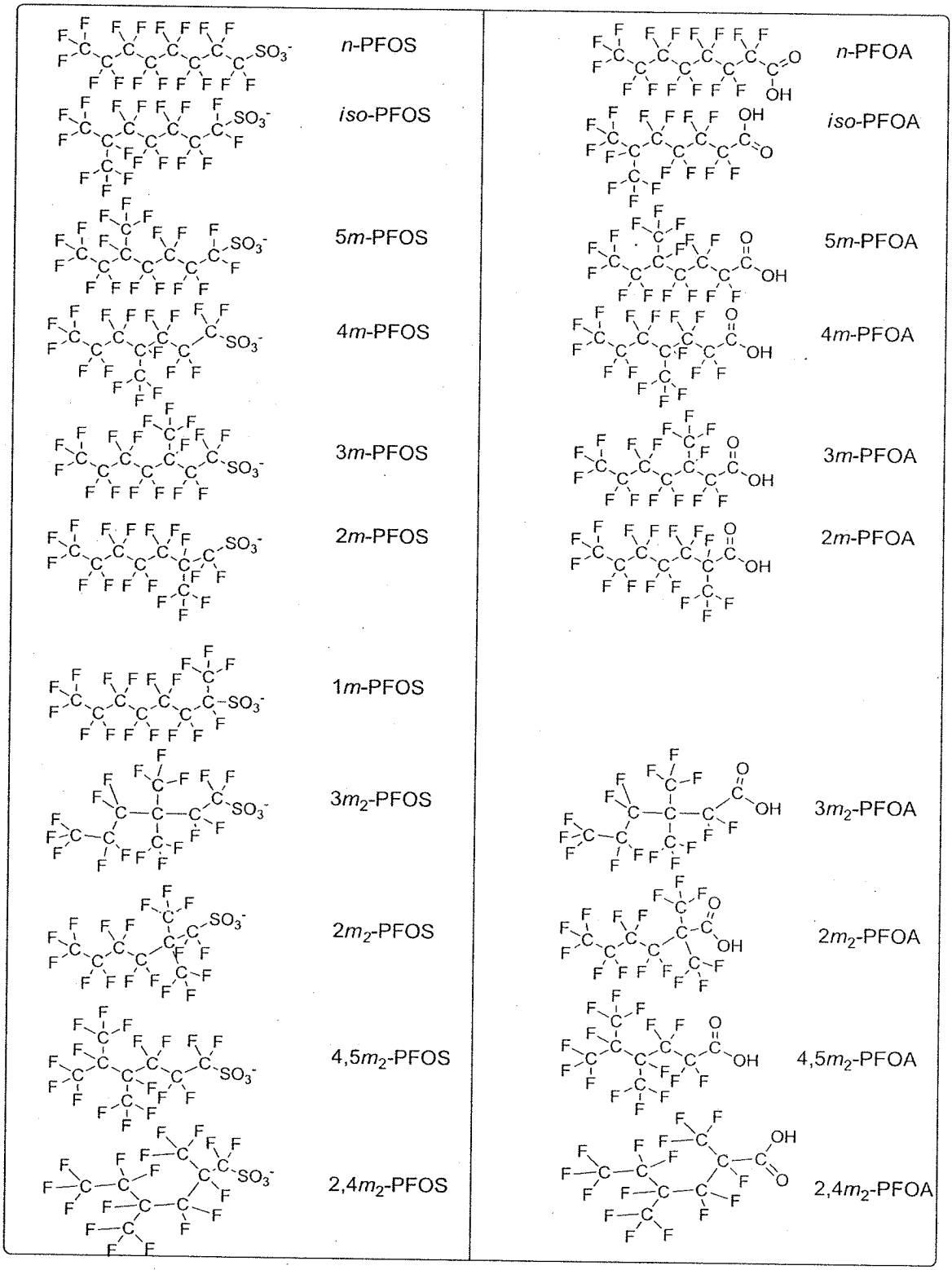
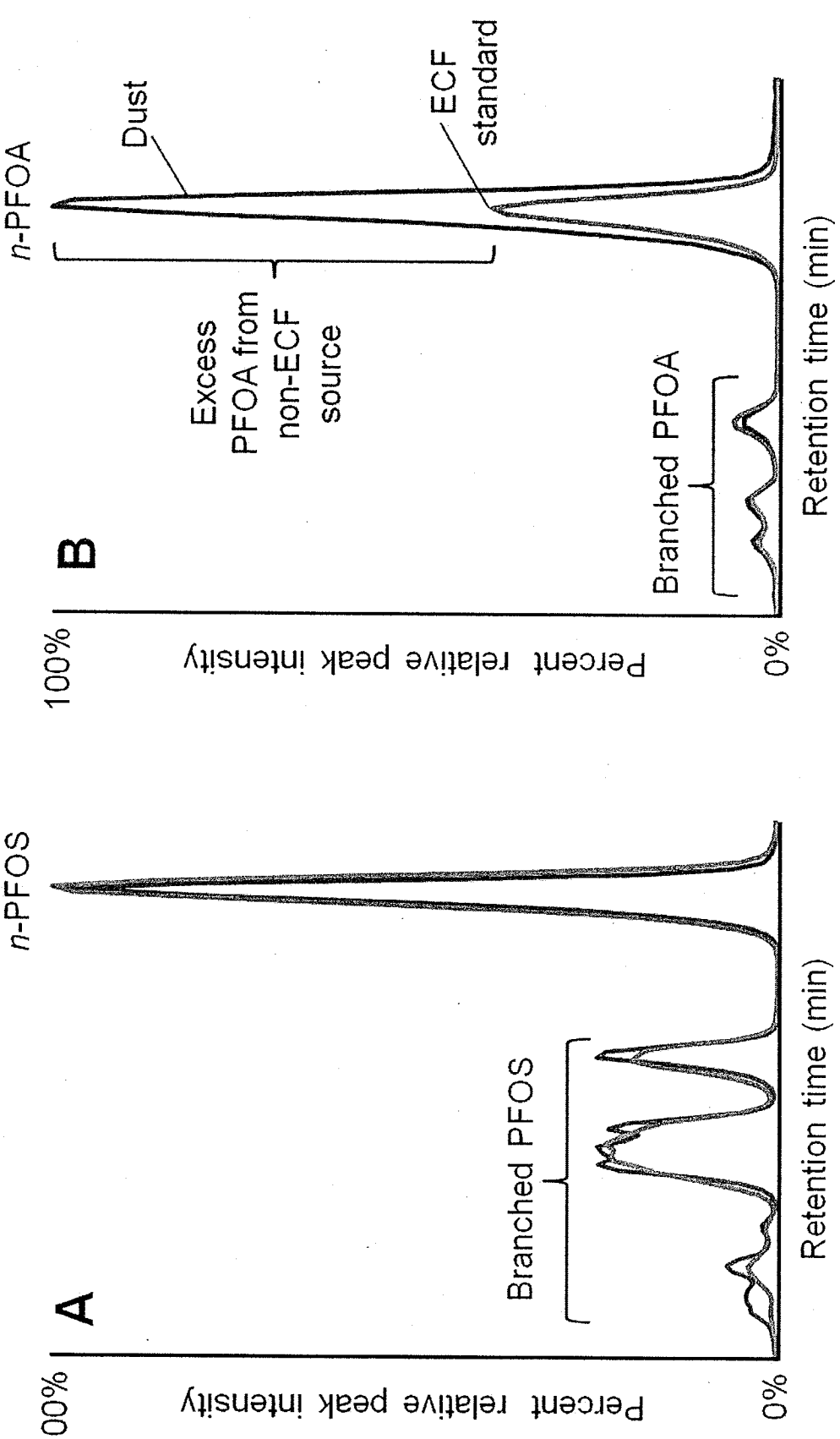


Supplemental Material, Figure 1. Trend in the geometric mean concentrations of PFOA and PFOS in the blood of Americans for the 10 year period from 1999 to 2008. PFOS has consistently declined, whereas PFOA has not declined since 2003/2004 and may be increasing. Data for the first 3 NHANES surveys are published data by the CDC, while the geometric means for 2007-2008 were calculated (after weight adjustment) using SAS data files available on the website of the National Center for Health Statistics of the CDC.



Supplemental Material, Figure 2. Structures of PFOS and PFOA linear and branched isomers. Note that 1*m*-PFOA does not exist. For geminal diperfluoromethyl-PFOS and geminal diperfluoromethyl-PFOA isomers, only the $3m_2$ and $2m_2$ are shown, although other such structures exist. Similarly for non-geminal diperfluoromethyl PFOS and PFOA only the 4,5 m_2 and 2,4 m_2 are shown, but other structures exist. In the isomer specific analysis, all the diperfluoromethyl PFOS isomers are grouped together and labeled as Σm_2-PFOS. Other nomenclature systems have been proposed (Rayne et al. 2008).



Supplemental Material Figure 3. Isomer profiles in a house dust sample for (A) PFOS (*m/z* 499/80) and (B) PFOA (*m/z* 413/369). Red traces represent the isomer profile in a 3M ECF standard, while the blue trace represents the isomer profile in a dust sample. Each profile was normalized to the response of the branched isomers, such that the relative amount of linear isomer in each case is easily compared.

Supplemental Material, Table 1. Spike and recovery of native PFCs in serum and dust. Experiments were done in triplicate in each case and figures presented here represent arithmetic means.

PFCs	Calf serum spiked at 0.5 ng/ml		Calf serum spiked at 10 ng/ml		Dust spiked at 60 ng/g	
	Mean % Recovery ±S.D	Range	Mean % Recovery ±S.D	Range	Mean % Recovery ± S.D	Range
PFBS	93.2 ± 16.9	79.4-128	-	-	71.7 ± 8.1	64.0 - 83.0
PFHxS	79.0 ± 13.0	61.8-94.8	91.8 ± 12.5	75 – 112	90.2 ± 12	72.1 - 105
PFHpS	95.5 ± 15.1	76.8-123	-	-	92.3 ± 27	46.8 - 116
PFOS	82.7 ± 16.8	52.0-99.3	93.1 ± 11.9	82 – 117	107 ± 14	89.9 - 121
PFDS	109 ± 8.7	93.3-120	64.0 ± 8.2	52 – 74	32.2 ± 15	14.7 - 48.9
PFBA	-	-	-	-	74.3 ± 8.9	60.1 - 84.7
PFPeA	-	-	-	-	83.7 ± 11	65.7 - 95.0
PFHxA	94.2 ± 9.3	78.3-108	-	-	128 ± 9	119 - 141
PFHpA	78.1 ± 10.7	66.2-97.4	-	-	97.9 ± 21	72.6 - 130
PFOA	82.5 ± 9.4	67.1-99.1	95.3 ± 15.5	68 – 129	83.0 ± 5.3	74.6 - 88.5
PFNA	94.4 ± 5.4	84.7-102	94.8 ± 8.3	74 – 101	93.1 ± 20	72.0 - 117
PFDA	81.9 ± 7.6	71.6-93.8	96.9 ± 4.6	91 – 106	102 ± 9	92.1 - 112
PFUnA	92.8 ± 7.6	81.6-106	93.8 ± 10.4	78 – 116	107 ± 11	95.1 - 118
PFDoA	102 ± 4.9	97.3-113	93.3 ± 5.8	83 – 101	95.6 ± 6.3	91.3 - 106
PFTrA	122 ± 6.9	115.0-133	54.0 ± 11.0	33 - 70	79.2 ± 17	59.9 - 106
PFTA	105 ± 7.4	97.2-117	-	-	42.1 ± 24	10.2 - 76.3
FOSA-M	108 ± 7.6	92.3-116	-	-	42.7 ± 4.9	38.2 - 50.9

Supplemental Material, Table 2. Summary statistics of total PFC concentrations (ng/g, non isomer specific) in house dust samples (n=18 unless otherwise noted). To compute the descriptive statistics, values less than limit of detection (LOD) have been replaced by LOD/2.

	Min	Max	Median	Mean	Geometric Mean	% above LOD
			Perfluoroalkyl sulfonates			
PFBS	<0.5	48	<0.5	6.1	0.7	28
PFHxS	2.9	1300	14	140	21	100
PFHpS	<0.5	46	<0.5	4.1	0.6	22
PFOS	<0.5	1300	37	180	39	94
PFDS	<0.5	5.1	2.1	2.2	1.8	94
			Perfluoroalkyl carboxylates			
PFBA	<0.5	42	2.6	9.2	3.6	94
PFPeA	<0.5	93	5.2	17	4.9	83
PFHxA	2.3	390	35	77	33	100
PFHpA	1.4	320	21	55	19	100
PFOA	4.3	820	38	120	50	100
PFNA	1.4	220	15	44	18	100
PFDA	1.7	250	15	44	16	100
PFUA	<0.5	240	6.1	31	8.0	94
PFDoA	1.4	160	10	36	13	100
PFTrA	<0.5	67	2.4	9.9	2.3	78
PFTA	<0.5	24	3.3	6.5	3.3	94
			Perfluoroalkyl sulfonamides			
PFOSA	<0.5	<0.5	<0.5	<0.5	0.3	0
NMeFOSA (n=16)	1.2	13.8	2.3	3.0	2.5	100
NEtFOSA (n=16)	<0.06	2.8	0.15	0.55	0.14	50
NMeFOSAA	<0.5	440	1.2	36	2.3	50
NEtFOSAA	3.2	240	27	58	32	100
NMeFOSE (n=16)	15	910	49	152	65	100
NEtFOSE (n=16)	<0.02	190	10	14	5.3	88

Supplementary Material, Table 3. Descriptive statistics of the percentage of individual isomers of PFOS and PFOA in house dust samples (N=18), and mean (n=3 injections) of the 3M ECF standard.

	PFOS							**PFOA**						
	Linear	*iso*	5*m*	4*m*	3*m*	1*m*	$\sum m_2$	Linear	*iso*	5*m*	4*m*	3*m*	$\sum m_2$	*tb*
Mean	69.4	17.6	4.55	0.61	5.08	0.81	2.13	82.0	5.54	4.77	3.12	3.21	0.80	0.56
SD[a]	4.81	3.60	1.01	0.16	1.78	0.77	0.68	11.2	3.28	3.57	3.01	1.77	0.59	0.4
Median	70.7	16.2	4.33	0.58	5.1	0.58	2.00	84.2	4.99	4.21	2.69	3.37	0.68	0.55
Min	53.4	14.8	3.20	0.42	<LOD[b]	<LOD	1.34	59.0	0.46	<LOD	<LOD	0.19	0.05	0.03
Max	73.5	27.6	7.73	1.14	9.65	2.26	4.31	98.6	12.2	11.6	8.15	6.17	2.38	1.62
Mean ECF Std	70.2	18.1	4.83	0.58	4.18	0.46	1.69	73.4	9.05	7.35	5.80	2.98	0.74	0.67

[a] The low relative standard deviations (SDs) associated with the mean percentage of PFOS isomers in the 18 dust samples suggest a similar ECF source, contrasted to the high SD for linear PFOA, suggesting a mixture of ECF and telomer sources.
[b] LOD-Limit of detection.

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J Agric Food Chem. 2011 Sep 19. [Epub ahead of print]

Perfluorinated Compounds in Human Blood, Water, Edible Freshwater Fish and Seafood in China: Daily Intake and Regional Differences in Human Exposures.

Zhang T, Sun H, Lin Y, Wang L, Zhang X, Liu Y, Geng X, Zhao L, Li F, Kannan K.

Abstract

Despite the growing public interest in perfluorinated compounds (PFCs), very few studies have reported the sources and pathways of human exposure to these compounds in China. In this study, concentrations of 10 PFCs were measured in human blood, water (tap water and surface water), freshwater fish and seafood samples collected from China. Based on the data, we calculated daily intakes of PFCs, regional differences in human exposures, and potential risks associated with ingestion of PFCs from diet, drinking water, and indoor dust for the Chinese population. Perfluorooctane sulfonate (PFOS) was the most predominant PFC found with a mean concentration of 12.5 ng/mL in human blood from Tianjin, and 0.92 ng/g wet wt in freshwater fish and seafood; perfluorooctanoic acid (**PFOA**) was the major PFC found in drinking water at a concentration range of 0.10 to 0.92 ng/L. The estimated daily intake of PFOS and **PFOA** via fish and seafood consumption (EDIfish&seafood) ranged from 0.10 to 2.51, and 0.13 to 0.38 ng/kg bw/d, respectively, for different age groups (i.e., toddlers, adolescents and children, and adults) from selected locations (i.e., Tianjin, Nanchang, Wuhan, Shenyang). The EDIfish&seafood of PFCs decreased ($p < 0.05$) with age. The estimated daily intake of PFOS and **PFOA** via drinking water consumption (EDIdrinking water) ranged from 0.006 to 0.014, and 0.010 to 0.159 ng/kg bw/d, respectively. Comparison of EDIfish&seafood, and EDIdrinking water values with the modeled total dietary intake (TDI) of PFCs by adults from Tianjin, Nanchang, Wuhan, and Shenyang showed that contributions of fish and seafood to TDI of PFOS varied depending on the location. Fish and seafood accounted for 7%, 24%, 80%, and 84% of PFOS intake in Nanchang, Shenyang, Wuhan, and Tianjin, respectively, suggesting regional difference in human exposure to PFOS. Drinking water was a minor source of PFOS (< 1%) exposure in adults from all the study locations.

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EXHIBIT C-65

Toxicology Reports 2 (2015) 939–949

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Evaluation of chronic toxicity and carcinogenicity of ammonium 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)-propanoate in Sprague–Dawley rats

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PPARα agonist

ABSTRACT

Ammonium 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)-propanoate, developed for use as a polymerization processing aid in the manufacture of fluoropolymers, was tested for its potential chronic toxicity and carcinogenicity in a 2-year oral dosing study in Sprague–Dawley rats. Male rats were given daily doses of either 0, 0.1, 1 or 50 mg/kg; females were given either 0, 1, 50 or 500 mg/kg. Body weights, food consumption and clinical signs were monitored daily; clinical pathology was conducted at designated intervals and animals were given a complete pathological evaluation after 12 months and 24 months of dosing. Normal survival was seen in all groups, no abnormal clinical signs were seen, and body weight gain was reduced only in female rats at 500 mg/kg. Both sexes at the high dose had mild decreases in red cell mass which were somewhat more pronounced in females. Clinical pathology indicative of liver injury was present in males that received 50 mg/kg and correlated with histomorphological liver changes that included both hypertrophic and degenerative/necrotic lesions. Similar histomorphological lesions were seen in the livers of females at 500 mg/kg. Previous shorter term toxicity studies have identified this chemical as a PPARα agonist and the finding of benign tumors of the liver, pancreas and/or testes in males at 50 mg/kg and females at 500 mg/kg is consistent with the rat response to peroxisome proliferators and is of questionable human relevance. Changes in the kidney, tongue, and stomach were observed only at the highest dose of 500 mg/kg in females. The no-observed-adverse-effect-level in this study lies between 1 and 50 mg/kg for males and between 50 and 500 mg/kg for females.

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1. Introduction

Ammonium 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)-propanoate (CAS 62037-80-3) is a white/colorless solid with a sublimation point of 130–140 °C, a decomposition point of 150–160 °C, a density of approximately 1.7 g/cm^3 at 20 °C, and a very low vapor pressure of approximately 0.01 Pa at 20 °C [10]. The chemical has been developed for use as a polymerization processing aid in the manufacture of fluoropolymers. The acute toxicity profile includes both an oral LD50 of 1750 and 3129 mg/kg in male and female rats, respectively, and 1030 mg/kg in mice (females). The dermal LD50 in rats is greater than 5000 mg/kg. By the inhalation route, the chemical has an acute LC50 of greater than 5200 mg/m^3 in rats exposed for 4 h. As tested in rabbits, it is highly irritating to the eye but is not a skin irritant nor is it a sensitizer as tested by the mouse local lymph node assay. The material is not genotoxic based on a battery of tests including Ames and chromosome aberration in-vitro studies and mouse micronucleus, mouse bone marrow chromosomal analysis and rat unscheduled DNA synthesis in-vivo studies [10].

The compound is rapidly and completely absorbed following oral administration in both rats and mice, is not metabolized, and is eliminated almost exclusively in the urine. In the cynomolgus monkey given a single intravenous dose, the chemical was rapidly eliminated, no sex differences were seen, and a biphasic pattern of elimination was present. Based on oral studies in rats and mice, and intravenous studies in rats and primates, elimination of the test substance is most similar and more rapid in primates and rats, with mice having a comparatively longer clearance time. Clearance

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times in rats and primates are generally less than 12–24 h while those in mice are 60–140 h. Plasma elimination kinetics were similar following both single and multiple dose studies indicating a low propensity for accumulation in the body. Based on the pharmacokinetic curve following an intravenous dose of the compound, male rats have a slightly slower elimination rate than females, resulting in males having a higher plasma concentration than females after the same dose [11].

In repeated dose toxicity studies, the test material induces hepatic peroxisomal β-oxidation activity and produces microscopic changes in the livers of rodents consistent with the activity of peroxisome proliferators. Thus, the test material is a member of a class of compounds known as PPARα agonists, which include endogenous long chain fatty acids, as well as many industrial chemicals and pharmaceuticals. These compounds produce effects in the liver to which rodents have been shown to be more sensitive than other species, including humans. In a series of repeated oral dosing studies of 28–90 days duration in rats and mice, the sentinel effects of this chemical were consistent with those of a PPARα agonist [12,13]. These included increased liver beta-oxidation activity, increased liver weights, microscopic hepatocellular hypertrophy, and clinical pathology changes of decreases in red cell parameters, changes in serum proteins, and decreases in serum lipids.

Specifically, in a 90-day repeated dose toxicity study in which male rats were given daily gavage doses of either 0.1, 10, or 100 mg/kg, findings included most of the clinical pathology and anatomic pathology effects noted above, as well as increased kidney weights [13]. Similar finding were seen in female rats given daily gavage doses of either 10, 100, or 1000 mg/kg. These included kidney weight increases at 10 mg/kg, PPARα-related clinical chemistry changes at 100 mg/kg, and red blood cell effects, increased liver weights, and hepatocellular hypertrophy at 1000 mg/kg. Findings similar to these were seen in an earlier 28-day dosing study with liver effects seen in males following dosing at 3 mg/kg and in females at 300 mg/kg [12]. These studies formed the basis for dose level selection for the chronic toxicity/carcinogenicity study reported here.

To characterize the chronic toxicity profile and to evaluate the potential carcinogenic effects of this chemical, a 2-year chronic toxicity and carcinogenicity study was conducted in rats. This paper covers the information derived from this experiment. The results of this study are reported herein and will be compared to those found in other chronic/carcinogenicity studies with similar fluorochemicals to place the findings in perspective.

2. Materials and methods

2.1. Chemicals

The ammonium salt of 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)-propionic acid (84%; CAS number 62037-80-3; $C_6H_4F_{11}NO_3$; molecular weight: 347) was provided by E.I. du Pont de Nemours and Company (Wilmington, DE). The test article was an 84% aqueous solution and doses were adjusted accordingly and are expressed in terms of 100% test chemical. Formulations of the test article were prepared weekly and stored at room temperature.

2.2. Animals

All rat treatments, toxicity assessments and data acquisitions were performed at MPI Research, Inc. (Mattawan, Michigan). The protocol was reviewed and approved by the Institutional Animal Care and Use Committee (IACUC), in compliance with the Animal Welfare Act (AWA). Male and female CD® [Crl:CD(SD)] rats were received at approximately 5 weeks of age from Charles River Laboratories (Portage, Michigan). Rats were acclimated for 14 days prior to being randomly assigned to treatment groups using a standard, by weight, measured value randomization procedure. Rats assigned to study had body weights within ±20% of the mean body weight for each sex. The animals were pair-housed (same sex) in polyboxes with non-aromatic bedding in an environmentally-controlled room and were provided environmental enrichment. All animals were maintained in accordance with the Guide for the Care and Use of Laboratory Animals in the animal facilities at MPI Research, Inc. (American Association for the Accreditation of Laboratory Animal Care [AAALAC] accredited). Block Lab Diet® (Certified Rodent Diet #5002, PMI Nutrition International, Inc., Shoreview, MN, USA) was provided ad libitum. Tap water was available ad libitum via an automatic watering system. Room temperature and humidity controls were maintained to the maximum extent possible between 64 and 79 °C and 30–70%, respectively. A 12 h light/dark photoperiod was maintained.

2.3. Treatment

Four treatment groups of 80 rats/sex were formed. Control group males and females received deionized water (vehicle control). Male rats in test groups received either 0.1, 1 or 50 mg ammonium 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)-propanoate/kg body weight/day. Female rats in test groups received either 1, 50 or 500 mg ammonium 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)-propanoate/kg body weight/day. Ten animals/sex/group were designated for the 12 month interim necropsy. The remaining surviving animals were designated for the terminal necropsy.

Dose selection was based on a previous subchronic (90-day) toxicity study [13] and a previous 28-day study [12]. The vehicle and test article were administered once daily for up to 104 weeks in males and up to 101 weeks in females via oral gavage at a dose volume of 10 mL/kg/dose. The control group received the vehicle in the same manner as the treated groups. Individual doses were based on the most recent body weights. All animals were observed for morbidity, mortality, injury, and availability of food and water twice daily. Beginning at week 53, a third daily cageside observation was added. A detailed clinical examination, including a physical exam and palpation of masses, was performed weekly. Ophthalmoscopic examinations were conducted on all animals pretest and on all surviving animals prior to the interim and terminal necropsies.

2.4. Body weight and food consumption

Body weights were recorded weekly starting day 1 (prior to dosing) until week 14, and then every two weeks thereafter. Body weight changes were calculated and reported weekly the first 3 months (weeks 1–13), the first year (weeks 1–52), and for the entire study (weeks 1–102 for males and weeks 1–100 for females). Food consumption was recorded pretest (week – 1), weekly during the first 13 weeks, and then every two weeks starting on week 14. Food consumption was measured for the cage and divided by the number of surviving animals. Food consumption and efficiency were calculated weekly the first 3 months (weeks 1–13), the first year (weeks 1–52), and for the entire study (weeks 1–102 for males and weeks 1–100 for females).

2.5. Clinical pathology

Clinical pathology evaluations were conducted on 10 animals/sex/group at 3, 6 and 12 months (hematology and clinical chemistry) and 10 animals/sex/group at 6 and 12 months (coagulation and urinalysis). The first ten animals in each group were

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selected for bleeding and these same animals were used for all time points. Blood samples were collected via the vena cava or cardiac puncture after carbon dioxide inhalation. The order of bleeding was by alternating 1 animal from each dose group then repeating to reduce handling and time biases. Blood samples for peripheral blood smears were collected at 12 and 18 months and prior to terminal necropsy. The animals designated for a full clinical pathology evaluation had access to drinking water but were fasted overnight prior to scheduled sample collection. The animals designated for only blood smears had access to drinking water and food prior to sample collection. Urine was collected after animals were housed in stainless steel metabolism cages for at least 12 h.

Hematology parameters evaluated included: leukocyte count (total and absolute differential), erythrocyte count, hemoglobin, hematocrit, mean corpuscular hemoglobin, mean corpuscular volume, mean corpuscular hemoglobin concentration (calculated), absolute reticulocytes, platelet count, and platelet morphology. Coagulation parameters evaluated included prothrombin time and activated partial thromboplastin time.

Clinical chemistry parameters evaluated included alanine aminotransferase, alkaline phosphatase, sorbitol dehydrogenase, total protein, albumin, globulin and A/G (albumin/globulin) ratio (calculated), urea nitrogen, creatinine, total cholesterol, triglycerides, total bilirubin (with direct bilirubin if total bilirubin exceeded 1 mg/dL), aspartate aminotransferase, total bile acids, glucose, calcium, phosphorus, electrolytes (sodium, potassium and chloride), and gamma glutamyl transferase.

Urinalysis parameters evaluated included volume, specific gravity, pH, color and appearance, protein, glucose, bilirubin, ketones, blood, urobilogen, and microscopy of centrifuged sediment.

2.6. Necropsy and histopathological evaluation

Postmortem study evaluations were performed on all animals euthanized in extremis, animals found dead, and on all surviving animals scheduled and sacrificed at the interim (12 months) and terminal (101 and 104 weeks – approximately 24 months) necropsies. The animals were euthanized by carbon dioxide inhalation followed by exsanguination via the abdominal vena cava. At necropsy, external surfaces and abdominal, thoracic, and cranial cavities were examined for abnormalities including masses. Tissues from all organ systems (digestive, urinary, respiratory, cardiovascular, hematologic, nervous, endocrine, musculoskeletal, and reproductive systems, as well as skin and gross observations) were removed, examined, and, where required by protocol, placed in fixative (neutral buffered formalin, except for the eye [including the retina and optic nerve] and testes, which were fixed using a modified Davidson's fixative). Body weights and organ weights (brain, adrenals, heart, kidneys, liver, spleen, thyroid/parathyroid, and/or epididymides, testes, ovaries with oviducts, and uterus with cervix) were recorded for all surviving animals at the scheduled necropsies and appropriate organ weight ratios were calculated (relative to body and brain weights). Paired organs were weighed together. Microscopic examination of fixed hematoxylin and eosin-stained paraffin sections was performed on tissues by a board-certified veterinary pathologist. A four-step grading system was utilized to define gradable lesions for comparison between dose groups. For fatal and incidental neoplasms, the onset date was considered to be the fate date of the affected animal. For mortality independent neoplasms, the onset date was considered to be the first appearance of a related abnormality (e.g., abrasion, nodule, and/or swelling). A second board-certified pathologist performed a formal peer review of the histopathological findings and the results reported here represent the consensus.

2.7. Statistical analysis

For continuous data (body weight and weight gain, food consumption, hematology, coagulation, clinical chemistries, and organ weights), the statistical procedures were generally as follows: Levene's test [20] was used to assess homogeneity of group variances for each specified endpoint and for all collection intervals. If Levene's test was not significant ($p > 0.01$), a pooled estimate of the variance (mean square error) was computed from a one-way analysis of variance (ANOVA) and utilized by a Dunnett's comparison [9] of each treatment group with the control group. If Levene's test was significant ($p < 0.01$), comparisons with the control group were made using Welch's t-test [28] with a Bonferroni correction.

Intercurrent mortality data were analyzed using the Kaplan–Meier product-limit method. An overall test comparing all groups was conducted using a log-rank test [2]. Tumor incidence data were analyzed using both survival adjusted and unadjusted tests. The Cochran–Armitage trend test [1] was calculated and Fisher's exact test [29] was used to compare each treatment group with the control group. The survival adjusted test was conducted according to the prevalence/mortality methods described by Peto et al. [24].

All endpoints were analyzed using two-tailed tests with results of all pair-wise comparisons reported at the 0.05 and 0.01 significance levels.

3. Results

This experiment was conducted in accord with the Toxic Substance Control Act (TSCA) Good Laboratory Practice Standards. All system suitability tests, performance checks, and calibration standards met analysis acceptance criteria and the dose formulation samples, analyzed for homogeneity and concentration, met the criteria for acceptance based on accuracy and precision. On quality assurance check, no significant deviations from the protocol which could impact the quality or integrity of the study were found.

3.1. Survival

Mean survival data for male and female rats are illustrated in Fig. 1. The test substance had no effect on survival. Females were sacrificed during week 101, prior to scheduled termination at week 104, due to low survival in all female dose groups (especially control and 50 mg/kg groups). However, even though survival among all female groups was low, there were no statistically significant differences between groups as survival was essentially the same.

Seven of the 500 mg/kg early death females had test substance-associated papillary necrosis and inflammation of the kidney. All other causes of death/morbidity were considered incidental and common in rats of this strain and age.

3.2. In-life observations

There were no test substance-related clinical observations; all observations were transient or common in this species. In addition, there were no test substance-related increases in masses identified during physical examination nor were there any test substance-related findings at the interim or terminal ophthalmoscopic examinations.

The test substance had no effect on body weight, body weight gain, or food consumption and efficiency in males. These values were generally comparable to those of control animals throughout the study.

Exposure of female rats to 500 mg/kg produced reductions in body weight, body weight gain, and food efficiency. Mean body weight for this group was 13% below that for control animals at

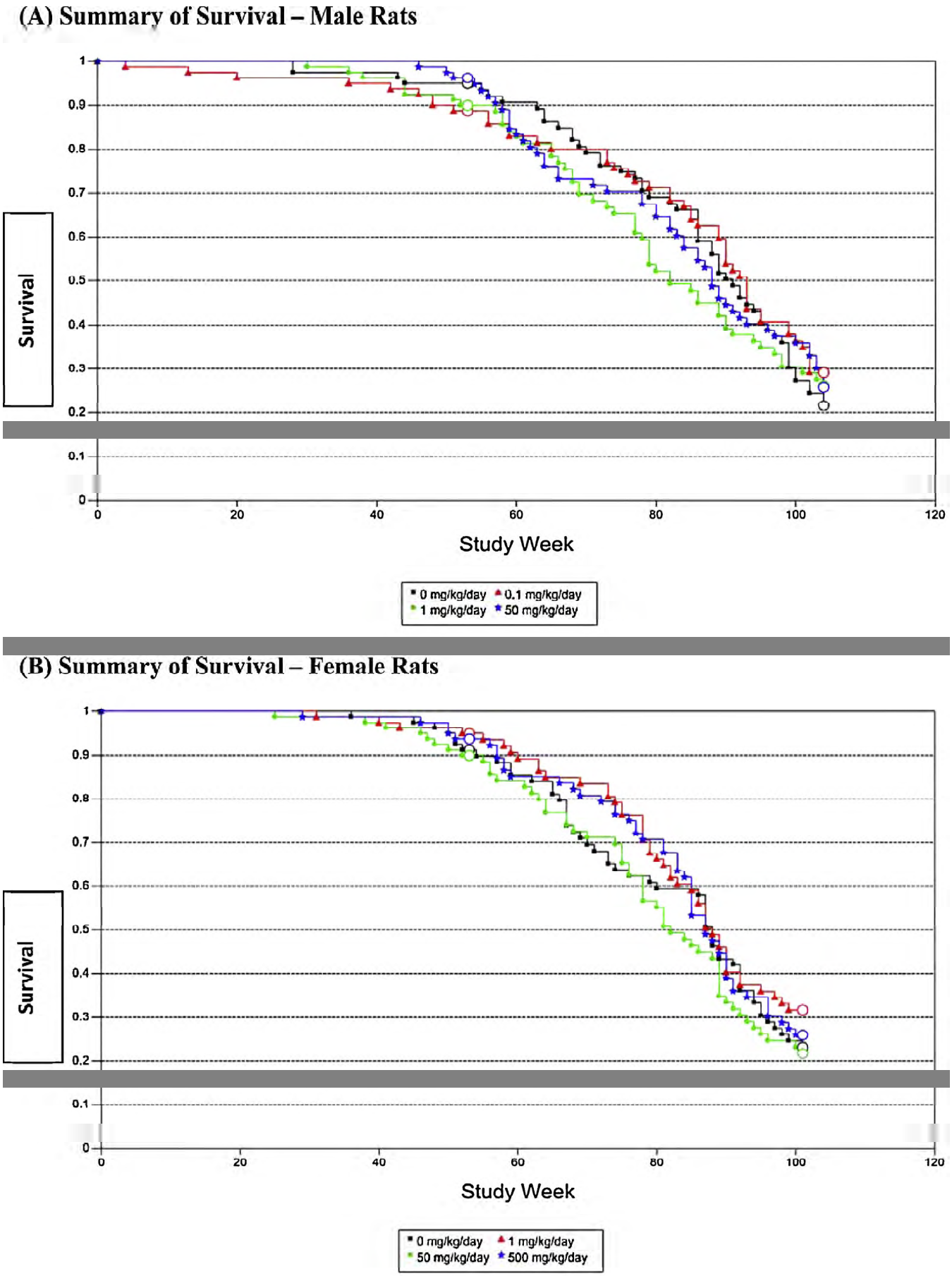

Fig. 1. Kaplan–Meier survival curves for rats treated with ammonium 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)-propanoate for 104 weeks (male) or 101 weeks (female). (A) Survival curve of male rats treated with either 0, 0.1, 1 or 50 mg/kg. $p > 0.05$. (B) Survival curve of female rats treated with either 0, 1, 50 or 500 mg/kg, $p > 0.05$.

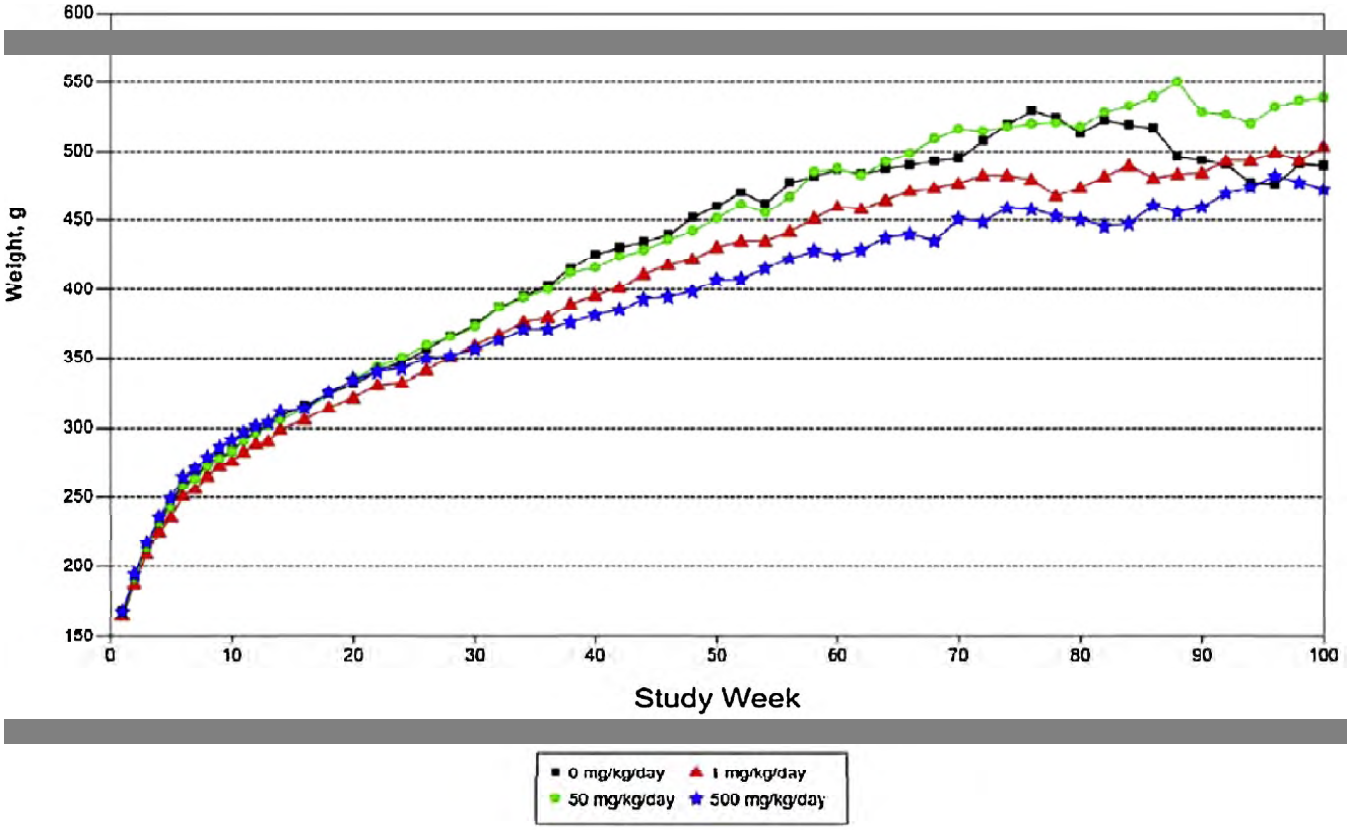

Fig. 2. Mean body weights for female rats treated with either 0, 1, 50 or 500 mg/kg ammonium 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)-propanoate for 101 weeks.

week 52 (statistically significant), but was comparable to controls at study termination (Fig. 2). Consequently, mean body weight gain was 20% below controls over weeks 1–52 in this group, but only slightly lower than controls over the entire 2 year study (not statistically significant). There were no effects on food consumption; the reduced body weight gain was associated with lower mean food efficiency over the first year (although food efficiency over the entire 2 year study was comparable to controls).

3.3. Clinical pathology

At the 3, 6, and 12 month intervals, the test substance caused mild but adverse decreases in red cell mass (erythrocytes, hemoglobin, and hematocrit; up to 28% below controls) in females receiving 500 mg/kg (Fig. 3). These changes were associated with an appropriate increase in reticulocytes (up to 106% above controls). There were no effects on erythrocyte morphology.

Statistically significant decreases in erythrocytes, hemoglobin, and hematocrit were also present in males receiving 50 mg/kg at the 3 and 6 month intervals. However, the decreases were small, transient (no statistically significant difference at 12 months), and did not induce a statistically significant increase in reticulocytes.

Increases in enzymes indicative of liver injury, including mild increases in alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, and sorbitol dehydrogenase (aspartate aminotransferase was not statistically significant) were observed at 12 months in males at 50 mg/kg (Fig. 4) and were correlated with microscopic findings of minimal cystic degeneration and minimal to mild focal necrosis in the liver. Minimal increases in alkaline phosphatase, unassociated with increases in other liver injury-specific enzymes, were also present in males at this dose level at 3 and 6 months. There were no test article-related liver enzyme changes in males receiving either 0.1 or 1 mg/kg or in females at any dose.

Test substance-associated increases in albumin (up to 16% above controls), decreases in globulin (up to 17% below controls), and increases in the albumin/globulin ratio were present in males at 50 mg/kg and females at 500 mg/kg at various intervals with variable statistical significance. Albumin was also statistically significantly increased in males receiving 1 mg/kg at 12 months (8% above controls) and globulin was statistically significantly decreased transiently (only at 6 months, 6% below controls) in females receiving 50 mg/kg. Minimal, statistically-significant increases in the albumin/globulin ratio were also present in the 1 mg/kg males and the 50 mg/kg females at various intervals.

A minimal diuresis was present at 6 and 12 months in females that received 500 mg/kg, as evidenced by statistically significant increases in urine volume and decreases in urine specific gravity. There were no changes in kidney-related chemistry parameters. This diuresis may be correlated to an increase in the incidence and severity of chronic progressive nephropathy (CPN) observed in this group at the 1-year sacrifice (Table 1).

There were no other test article related clinical pathology effects. All other mean and individual clinical pathology parameters were considered within an acceptable range for biological and procedure-related variation.

3.4. Organ weights and anatomic pathology observations

No test-substance associated anatomic pathology findings occurred in male rats administered either 0.1 or 1 mg/kg or in female rats administered either 1 or 50 mg/kg. Test substance-associated findings observed in males at 50 mg/kg and in females at 500 mg/kg are described in Section 3.4.1, 3.4.2 and 3.4.3. All

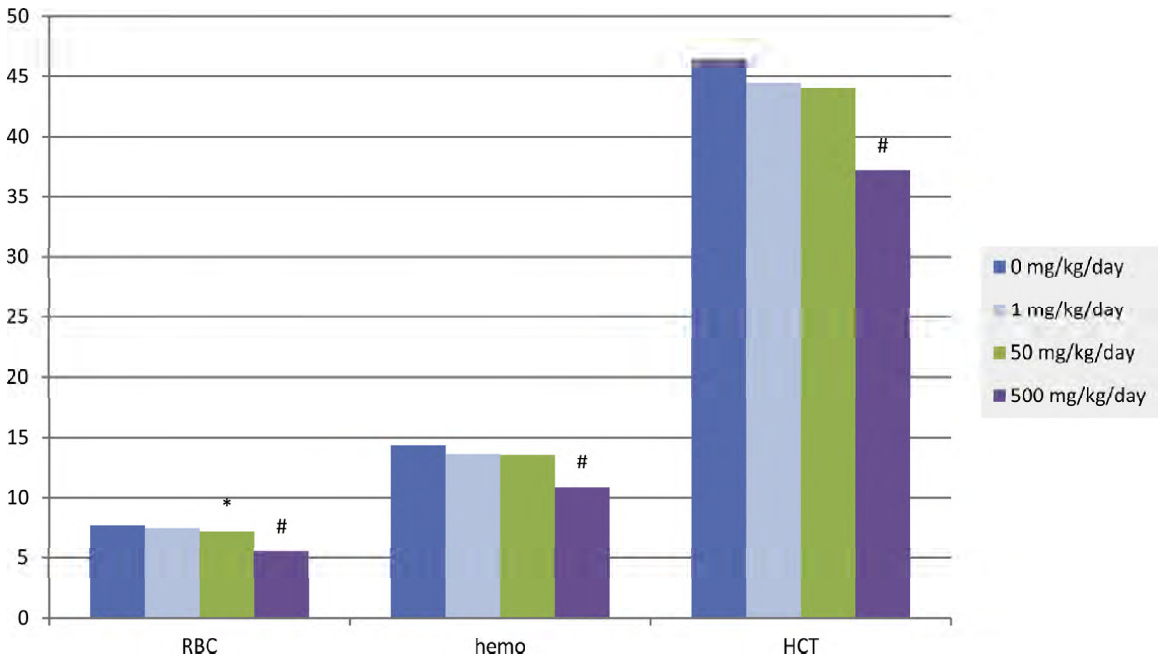

Fig. 3. Mean red cell mass parameters for female rats treated with either 0 (control), 1, 50 or 500 mg/kg ammonium 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)-propanoate for 12 months. RBC = erythrocytes ($10^6/\mu L$); hemo = hemoglobin (g/dL); HCT = hematocrit (%). * = Significantly different from control ($p < 0.05$); # = significantly different from control ($p < 0.01$), $N = 10$.

other organ weight, macroscopic and non-neoplastic and neoplastic microscopic observations were of the type typically seen in rats of this strain and age, and were considered incidental and not related to compound administration.

3.4.1. Organ weights and macroscopic findings

At the interim necropsy, test substance-associated organ weight effects were limited to increased liver weights (Table 1) in the 50 mg/kg male group (16% above controls relative to body weight) and in the 500 mg/kg female group (69% above controls relative to body weight). In females, liver weight changes correlated with centrilobular hypertrophy microscopically. In one 500 mg/kg female, the macroscopic finding of irregular surface of the kidney correlated with CPN in this animal and was likely test substance-related.

At the terminal sacrifice, no test compound-associated organ weight or macroscopic effects were observed in males. In females, test substance-associated organ weight changes were limited to increased liver weights (Table 1) at 500 mg/kg (43% above con-

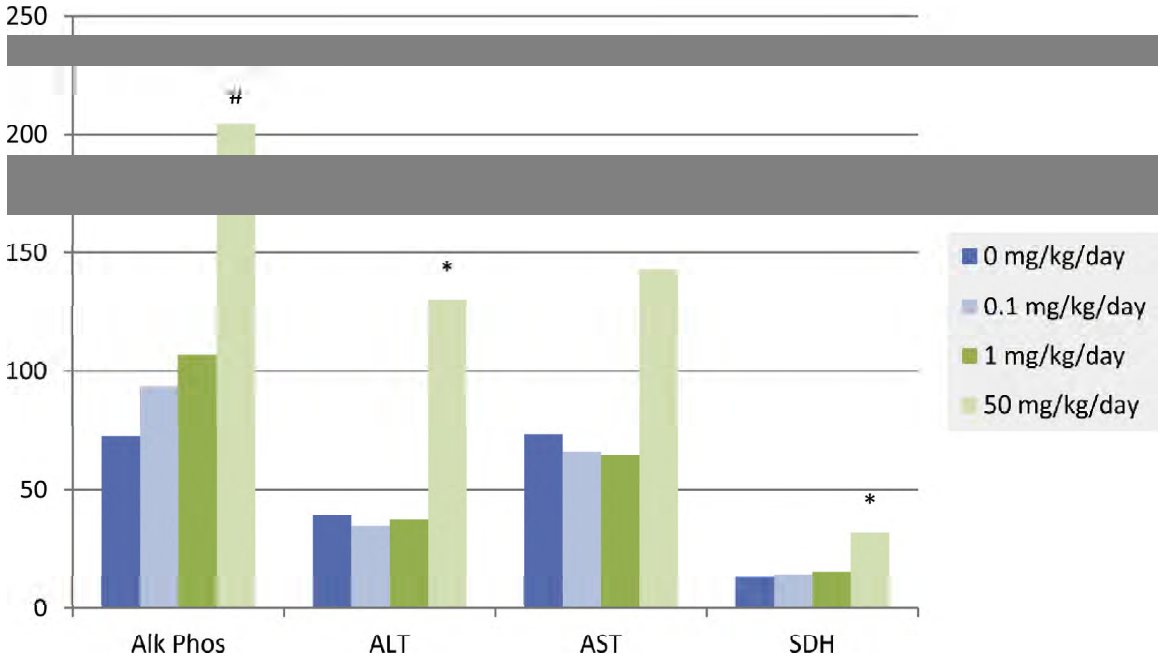

Fig. 4. Selected mean clinical chemistry parameters for male rats treated with either 0 (control), 0.1, 1 or 50 mg/kg ammonium 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)-propanoate for 12 months. Alk Phos = alkaline phosphatase (U/L); ALT = alanine aminotransferase (U/L); AST = aspartate aminotransferase (U/L); SDH = sorbitol dehydrogenase (U/L). * = Significantly different from control ($p < 0.05$); # = significantly different from control ($p < 0.01$), $N = 10$.

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Table 1
Mean liver weight parameters for male rats treated with either 0, 0.1, 1 or 50 mg/kg ammonium 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)-propanoate and female rats treated with either 0, 1, 50 or 500 mg/kg ammonium 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)-propanoate for 12 (interim sacrifice) and 24 (terminal sacrifice) months.

Mean liver weights at interim and terminal sacrifice in male and female rats								
Males								
	Interim sacrifice				Terminal sacrifice			
Dose level (mg/kg)	0	0.1	1	50	0	0.1	1	50
N	10	10	10	10	15	20	18	18
Absolute (g)	26.9 ± 4.9[*]	26.1 ± 4.3	25.5 ± 1.9	29.7 ± 5.0	24.3 ± 3.6	26.6 ± 6.4	24.1 ± 5.6	27.1 ± 4.8
%		−3%	−5%	10%		9%	−1%	12%
Liver/body weight (%)	3.2 ± 0.3	3.3 ± 0.3	3.3 ± 0.2	3.7 ± 0.4[#]	2.9 ± 0.4	3.3 ± 1.3	3.2 ± 0.7	3.2 ± 0.5
%		3%	3%	16%		14%	10%	10%
Females								
	Interim sacrifice				Terminal sacrifice			
Dose level (mg/kg)	0	1	50	500	0	1	50	500
N	10	10	10	10	16	22	15	18
Absolute (g)	13.2 ± 2.5	12.0 ± 1.6	14.8 ± 3.6	17.6 ± 2.6[#]	16.4 ± 3.8	15.8 ± 4.3	17.2 ± 3.0	23.2 ± 8.2[#]
%		−9%	12%	33%		−4%	5%	41%
Liver/body weight (%)	2.9 ± 0.3	3.0 ± 0.3	3.3 ± 0.4	4.9 ± 0.6[#]	3.5 ± 0.4	3.3 ± 0.7	3.3 ± 0.6	5.0 ± 0.9[#]
%		3%	14%	69%		−6%	−6%	43%

[#] Significantly different from control ($p < 0.01$). %Indicates difference from controls.
[*] Mean ± standard deviation.

trols relative to body weight). Macroscopic findings in this group included tan focus/foci in the liver (8/70 versus 1/70 in controls), mass/nodule in the liver (14/70 versus 0/70 in controls) and irregular surface of the kidney (16/70 versus 0/70 in controls). The organ weight and macroscopic observations in females were correlated to non-neoplastic and neoplastic histological observations at this dose.

3.4.2. Non-neoplastic histological findings

At the interim necropsy, non-neoplastic test substance-associated effects were present in the liver of males at 50 mg/kg and in the liver and kidneys of females at 500 mg/kg. In the liver of males administered 50 mg/kg, histological lesions included minimal focal cystic degeneration (3/10 versus 0/10 in controls) and minimal to mild focal necrosis (5/10 versus 1/10 in controls). In females, histological changes in the liver were limited to minimal to mild hepatocellular hypertrophy in all 10 females receiving 500 mg/kg. A slightly increased incidence and severity of CPN was also observed in interim sacrifice females at 500 mg/kg (Table 2).

At the terminal sacrifice, non-neoplastic test substance-associated effects were observed histologically at the highest dose level in the liver of both sexes (Table 3) and, additionally, in the kidney (Tables 4), glandular stomach (limiting ridge) and tongue of females at the highest dose (Table 5).

In 50 mg/kg males, histopathological effects were limited to the liver and included statistically significant increases in the incidences of focal cystic degeneration, centrilobular hepatocellular hypertrophy, and centrilobular hepatocellular necrosis. These changes were also present in 500 mg/kg females, in addition to low incidences of panlobular hepatocellular hypertrophy and individual cell hepatocellular necrosis (Table 3). Cystic degeneration was characterized by the presence of multilocular cystic spaces containing finely granular or flocculent material without endothelial or epithelial cells lining the spaces. Centrilobular hypertrophy, morphologically consistent with peroxisome proliferation, was characterized by hepatocytes with red granular cytoplasm sometimes containing small amounts of pigment compatible with lipofuscin. Centrilobular hepatocellular necrosis was typically of the coagulative type with strongly eosinophilic-staining cytoplasm and pyknotic nuclei. Panlobular hepatocellular hypertrophy was characterized by enlargement of hepatocytes throughout the entire liver. Individual cell necrosis was characterized by the presence of scattered single hepatocytes with features characteristic of apoptosis.

Kidney changes in females at 500 mg/kg included tubular dilation, edema of the renal papilla, transitional cell hyperplasia in the renal pelvis, tubular mineralization, renal papillary necrosis and CPN. Tubular dilation frequently occurred in an ascending pattern extending from the papilla to the outer cortex, while at other times it was present only in the papilla. Edema of the papilla was characterized by increased rarefaction or myxomatous change in the papillary interstitium, sometimes with polypoid protrusions from the lateral surface of the papilla. The edema and tubular dilation were often associated with hyperplasia of the transitional cell epithelium lining the papilla and pelvis. Small foci of tubular mineralization were often present and, in some animals, necrosis of the tip of the papilla was present.

In addition, in female rats given 500 mg/kg, statistically significant increases in hyperplasia of squamous epithelium were observed in the nonglandular stomach (limiting ridge only) and tongue (in association with subacute/chronic inflammation in the tongue).

A statistically significant increase (42/70 or 61%) in alveolar histiocytosis was present in females at 500 mg/kg and was at the upper end of the historical control range of 9.2–61.7% [22]. The increased incidence of this common background lesion may be secondary to aspiration of dosing formulation at this high concentration; however, a definitive mechanism for this increase could not be determined.

3.4.3. Neoplastic histological findings

Neoplastic test substance-associated effects were observed histologically at the highest dose level in the liver of females and, equivocally, in the pancreas and testes of males at the highest dose (Table 6).

Compound-related neoplastic changes occurred in the livers of females administered 500 mg/kg and included increased incidences of hepatocellular adenoma and carcinoma (Table 6). These tumors occurred in association with the degenerative and necrotic liver lesions observed at this dose as described above. Hepatocellular tumors and test substance-associated degenerative and necrotic lesions were not observed in females at lower doses and the incidences of hepatocellular tumors were similar in all male groups. In

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Table 2
Incidence of chronic progressive nephropathy in the kidneys of female rats treated with either 0 (control), 1, 50 or 500 mg/kg of ammonium 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)-propanoate for 12 months, $N = 10$.

Incidences and severity of chronic progressive nephropathy in the kidneys of female rats: interim sacrifice				
Dose level (mg/kg)	0	1	50	500
Nephropathy, chronic progressive	6	4	6	9
-Minimal	5	3	4	3
-Mild	0	1	2	6
-Moderate	1	0	0	0

Table 3
Test substance-associated non-neoplastic histological lesions in the liver of male rats treated with either 0, 0.1, 1 or 50 mg/kg ammonium 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)-propanoate and female rats treated with either 0, 1, 50 or 500 mg/kg ammonium 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)-propanoate for 24 months, $N = 70$.

Summary of test substance-associated non-neoplastic findings in the liver of male and female rats: terminal sacrifice				
Males				
Dose level (mg/kg)	0	0.1	1	50
Degeneration, cystic, focal	24	24	19	42*
Hypertrophy, hepatocyte, centrilobular	0	0	0	7*
Necrosis, hepatocytes, centrilobular	1	0	1	5*
Females				
Dose level (mg/kg)	0	1	50	500
Degeneration, cystic, focal	2	2	2	14*
Hypertrophy, hepatocyte, centrilobular	0	0	3	65*
Hypertrophy, hepatocyte, panlobular	0	0	0	3*
Necrosis, hepatocytes, centrilobular	1	1	4	7*
Necrosis, individual hepatocyte	0	0	0	3*

* Significantly different from control ($p < 0.05$).

males administered 50 mg/kg, a statistically significant increase in the combined incidence of pancreatic acinar cell adenomas and carcinomas was seen (Table 6), but neither the incidence of adenoma or carcinoma alone was statistically increased, although the incidence of carcinomas (2.9%) was slightly outside the historical range of 0–1.7% [21]. In addition, the incidence of acinar cell hyperplasia was not significantly different from controls in any treated male groups. However, based on the known PPARα agonist activity of the test compound, the marginal increase in pancreatic acinar cell tumors at this dose provides equivocal evidence of a test substance-associated effect. Incidences of proliferative acinar cell lesions in males receiving 0.1 and 1 mg/kg were similar to controls and no acinar cell adenomas or carcinomas were present in females at any dose.

The incidence of Leydig cell adenomas (11.4%) was increased above historical control ranges for this tumor (0–8.3%) [21] in males administered 50 mg/kg, although this increase was not statically significant compared to controls. In addition, a Leydig cell adenoma was present in 1 male at the interim necropsy in the 50 mg/kg group. The incidence of Leydig cell hyperplasia was also

Table 4
Test substance-associated non-neoplastic histological lesions in the kidney of female rats treated with either 0, 1, 50 or 500 mg/kg ammonium 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)-propanoate for 24 months, $N = 70$.

Summary of test substance-associated non-neoplastic findings in the kidneys of female rats: terminal sacrifice				
Dose level (mg/kg)	0	1	50	500
Dilation, tubular	4	2	5	28*
Edema, papilla	4	1	2	43*
Hyperplasia, transitional cell	6	3	12	33*
Mineralization, tubular	25	32	28	42*
Necrosis, papillary	0	0	0	16*
Nephropathy, chronic progressive	39	40	41	64*

* Significantly different from control ($p < 0.05$).

Table 5
Test substance-associated non-neoplastic histological lesions in the nonglandular stomach and tongue of female rats treated with either 0, 1, 50 or 500 mg/kg ammonium 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)-propanoate for 24 months, $N = 70$.

Summary of test substance-associated non-neoplastic findings in the nonglandular stomach and tongue of female rats: terminal sacrifice				
Dose level (mg/kg)	0	1	50	500
Stomach, nonglandular				
Hyperplasia, epithelial, limiting ridge	0	0	0	9*
Tongue				
Hyperplasia, squamous cell	2	8	4	13*
Inflammation, subacute/chronic	3	8	4	13*

* Significantly different from control ($p < 0.05$).

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Table 6
Test substance-associated neoplastic histological lesions in the liver, pancreas and testes of male rats treated with either 0, 0.1, 1 or 50 mg/kg ammonium 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)-propanoate and the liver and pancreas of female rats treated with either 0, 1, 50 or 500 mg/kg ammonium 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)-propanoate for 24 months, $N = 70$.

Summary of test substance-associated neoplastic findings in male and female rats: terminal sacrifice				
Males				
Dose level (mg/kg)	0	0.1	1	50
Liver				
Hepatocellular adenoma	1	2	1	1
Hepatocellular carcinoma	1	0	0	2
Pancreas				
Acinar cell adenoma	0	1	0	3
Acinar cell carcinoma	0	0	0	2
Combined adenoma/carcinoma	0	1	0	5*
Testes				
Leydig cell tumor	4	4	1	8
Females				
Dose level (mg/kg)	0	1	50	500
Liver				
Hepatocellular adenoma	0	0	0	11*
Hepatocellular carcinoma	0	0	0	4*
Pancreas				
Acinar cell adenoma	0	0	0	0
Acinar cell carcinoma	0	0	0	0
Combined adenoma/carcinoma	0	0	0	0

* Significantly different from control ($p < 0.05$).

increased above historical control range in this group at terminal sacrifice (also 0–8.3%; [22]; although again, this incidence was not statistically significant versus controls. However, comparison to within-study controls was complicated by the fact that controls had a relatively high incidence of Leydig cell hyperplasia (10%). Based on the above considerations and the known activity of PPARα agonists to produce Leydig cell hyperplasia and adenomas in rats, the relationship to the test compound for these lesions was considered equivocal in this study.

All other findings were considered to represent the spontaneous occurrence of neoplasms commonly seen in rats of this strain and age.

4. Discussion

Doses employed in this study were designed to and did meet the expectations stated below based on information gathered from previous shorter-term repeated dose studies. The high dose (50 mg/kg in males, 500 mg/kg in females) did produce adverse effects on a number of parameters without producing excessive toxicity which might affect survival. Seven females in the 500 mg/kg group died from papillary necrosis of the kidney but overall survival in this group was similar to that of the other test groups and the controls. The intermediate dose (1 mg/kg in males, 50 mg/kg in females) was selected to either produce slight to minimal effects on target tissues and organs (liver and kidney) and/or to be a no-observed adverse effect level (NOAEL), which it was found to be. The low dose (0.1 mg/kg in males, 1 mg/kg in females) was projected and also found to be a NOAEL in both sexes.

No unusual clinical signs were observed in any of the test groups during the course of the study. Body weights among females in the 500 mg/kg group were significantly reduced, being up to 20% lower than those of the controls. No body weight differences were seen among the male test groups. Food consumption in all test groups was unchanged; however, because of the weight gain depression, females in the 500 mg/kg group showed lower food efficiencies.

Females receiving 500 mg/kg had mild but adverse decreases in red cell mass parameters at all time points tested. These changes were associated with an appropriate increase in reticulocytes and were not accompanied by changes in erythrocyte morphology. Similar decreases in red cell mass parameters were also present in males receiving 50 mg/kg at the 3 and 6 month intervals. However, in males, the decreases were small, transient (there was no statistically significant difference at 12 months), did not induce a statically significant increase in reticulocytes, and values in individual animals were similar to controls. Thus, the changes observed in 50 mg/kg males were considered to be related to treatment but were not considered adverse. This collection of findings suggests red cell loss or hemolysis, although the exact mechanisms involved are unknown. Peroxisome proliferators have been shown to affect iron metabolism, and this mechanism has been hypothesized to be the possible cause of the mild anemia sometimes seen in humans and rats that receive these compounds [14,15]. No changes in leukocyte numbers or differential counts were seen and coagulation parameters were normal in all test groups.

Clinical chemistry evaluations among males receiving 50 mg/kg revealed mild but adverse increases in enzymes indicative of liver injury, including alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, and sorbitol dehydrogenase. These enzyme changes correlated with microscopic findings in the liver which consisted of minimal cystic degeneration and minimal to mild focal necrosis. Transient, minimal increases in alkaline phosphatase, unassociated with increases in other liver injury-specific enzymes, were also present in males at this dose level at 3 and 6 months; however, this increase was likely due to hepatic enzyme induction as the test substance has been demonstrated previously to increase total P450 enzyme activity in male rats at 30 mg/kg [12]. These liver enzyme changes were not seen in the male groups receiving either 0.1 or 1 mg/kg or in any of the female test groups.

In addition, a small increase in serum albumin and a decrease in serum globulin were seen in males in the 50 mg/kg group and females in the 500 mg/kg group which led to increases in the albumin/globulin ratio. Slight changes in these serum protein components were also seen in males given 1 mg/kg and females given 50 mg/kg. The test chemical is a peroxisome proliferator and the pattern of change in serum proteins is a well-established

response to PPAR activation [8]. Peroxisome proliferators are anti-inflammatory, producing decreases in acute phase proteins (which contribute to the globulin fraction) and increases in the negative acute phase protein, albumin. No adverse biological outcomes have been associated with such changes in these serum proteins. Therefore, the statistically significant changes in albumin/globulin ratio in these groups were considered to be test article-related but nonadverse based on the minimal nature of the changes and lack of association with known adverse outcomes.

The minimal diuresis observed in females that received 500 mg/kg at 6 and 12 months was not associated with changes in kidney-related chemistry parameters. This diuresis may be correlated to an increase in the incidence and severity of CPN observed in this group at the 1-year sacrifice (Table 1).

Liver weights were increased in high dose male and female rats at the interim sacrifice and in high dose females only at terminal sacrifice. Microscopic evaluation of the liver revealed changes only in the high dose males and females. These changes occurred at both the terminal sacrifice and, in a less progressed form, at the interim sacrifice and consisted of microscopic observations including focal cystic degeneration, centrilobular (and panlobular in females only) hepatocellular hypertrophy, centrilobular hepatocellular necrosis, and individual cell necrosis (females only). In addition, the incidences of both adenoma and carcinoma in the liver exceeded the historical control range in females administered 500 mg/kg (only a few hepatocellular tumors occurred in males with the incidence being essentially the same between the controls and the test groups) and the increased incidences of hepatocellular tumors in females occurred in association with degenerative/necrotic liver changes. This battery of liver changes is consistent with PPARα activation.

Other non-neoplastic microscopic observations included findings in the kidneys of females at 500 mg/kg at both the interim (in a less severe form) and terminal sacrifices, and included tubular dilation, edema of the renal papilla, transitional cell hyperplasia in the renal pelvis, tubular mineralization, renal papillary necrosis, and CPN. In addition, the nonglandular stomach (limiting ridge only) and the tongue had statistically significantly increased incidences of hyperplasia of squamous epithelium in females at 500 mg/kg at the terminal sacrifice only. The lesion in the tongue was associated with subacute/chronic inflammation.

In males administered 50 mg/kg, the incidences of pancreatic acinar cell adenoma/carcinoma combined, but not adenoma or carcinoma alone, were statistically significantly increased and the incidence of carcinoma was slightly outside the historical control range. Since pancreatic acinar cell hyperplasia and adenoma in rats occur along a continuum, the incidence of acinar cell hyperplasia would have been expected to be increased if a test-article related increase in acinar cell adenoma was found. However, the incidences of acinar cell hyperplasia were not significantly different from controls in any of the treated male groups. Based on these considerations, and the known PPARα agonist activity of the test article, the marginal increase in pancreatic acinar cell tumors in this group provides equivocal evidence of a test article-related effect.

The incidences of Leydig cell adenoma of the testes were increased above historical control range in the 50 mg/kg group at terminal sacrifice, although this finding was not statistically significant. In addition, a single Leydig cell adenoma was also present in 1 male from this group at the interim sacrifice. Incidences of Leydig cell hyperplasia were also elevated in this group above historical control range, although this, too, was not statistically significant compared to controls. Since PPARα agonists are known to produce proliferative Leydig cell lesions (hyperplasia and adenoma) in the testes of rats, a relationship to treatment for these findings in the 50 mg/kg group cannot be ruled out.

The test chemical belongs to a class of compounds known as peroxisome proliferators (PPARα agonists) which are known to produce liver, pancreatic, and testicular tumors in rats and liver tumors in mice [3,16]. However, these compounds have not been shown to be carcinogenic in other species including humans [16,7]. Based on the extensive research into the comparative biology of peroxisome proliferator-induced hepatic carcinogenesis, the induction of liver tumors in rodents by non-genotoxic peroxisome proliferators (this compound was shown to be inactive in a battery of genotoxicity assays) is not considered relevant to humans [23,7,17]. While there is less definitive mechanistic data on the role PPARα plays in the induction of pancreatic acinar cell tumors in rats, the available data involving altered bile flow and increased cholecystokinin suggests that this mode of action is also likely to be non-relevant for humans [16]. While less robust, research considering comparative biology and mechanism of action of Leydig cell tumor induction in rodents by a wide variety of chemical classes suggests these tumors most likely have low relevance to humans [25,6].

The findings of this study with this fluorochemical polymerization processing aid can be compared to the results from similar studies with other fluorochemical polymerization processing aids (and PPARα agonists). The perfluorinated C6 chain chemical, perfluorohexanoic acid (PFHxA), showed no evidence of an adverse effect in male rats given oral doses of up to 100 mg/kg for 2 years [18]. Females given either 5, 30, or 200 mg/kg showed a dose-related decrease in survival and, at 200 mg/kg, had kidney lesions characterized by papillary necrosis and tubular degeneration. No evidence for a tumorigenic response was found. For a sulfonated fluorochemical, perfluorooctane sulfonate (PFOS), when fed in the diet of rats for 2 years at doses equivalent to approximately 0.02, 0.1, 0.2, and 1 mg/kg, the primary organ affected was the liver with changes consisting of hepatocellular centrilobular hypertrophy, eosinophilic granules, and vacuolation [27]. Neoplastic lesions consisted of an increase of hepatocellular adenomas in male rats; females showed no increase in the incidence of neoplasia. With the ammonium salt of perfluorooctanoic acid (APFO), when fed for 2 years to male and female rats at doses approximately equivalent to 1.5 and 15 mg/kg, the liver was the target organ with an increase in the incidence of hepatocellular hypertrophy, monocellular infiltration, and hepatocellular vacuolation [5]. The neoplastic changes in this study were restricted to Leydig cell adenomas of the testes. In a follow-up mechanistic study, male rats treated at 300 ppm of APFO for 2 years (equivalent to a daily dose of 15 mg/kg) showed increased tumor incidences in the liver (hepatocellular adenomas), pancreas (acinar cell adenomas), and testes (Leydig cell adenomas) [4].

The neoplastic changes found with this fluorochemical are consistent with many of those seen with the compounds above and with other peroxisome proliferating chemicals, such as clofibrate [26], HCFC-123 [19], gemfibrozil, and diethyl-hexyl phthalate [6], when tested in rodents. Although hepatocellular adenomas were not identified in males in this study, the neoplastic pattern is similar to that seen in rats administered APFO but was seen at a dose somewhat higher (50 versus 15 mg/kg). In female rats, no increase in liver neoplasia was reported with APFO while this chemical produced an increase in both adenomas and carcinomas, but the response was seen only when the dose was considerably higher (500 versus 15 mg/kg) than the dose used in the APFO study.

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EXHIBIT C-66

Fact Sheet: Draft Toxicity Assessments for GenX Chemicals and PFBS

Federal, state, tribal, and local governments are working together to address per- and polyfluoroalkyl substances (PFAS) in the environment. PFAS are man-made chemicals used in a wide range of products because of their ability to repel water, grease, and oil. While PFOA and PFOS are the two most extensively produced and studied chemicals in the group, EPA is asking for public comment on draft toxicity assessments for GenX chemicals and perfluorobutane sulfonic acid (PFBS) to increase the amount of information the public has on other PFAS. When issued, the toxicity assessments can be used along with exposure information and other important considerations to assess potential health risks to determine if, and when, it is appropriate to action to address these chemicals.

Questions and Answers

What are PFAS, including PFBS and GenX chemicals?

PFAS: Per- and polyfluoroalkyl substances (PFAS) are a group of man-made chemicals that have been in use since the 1940s, and are (or have been) found in many consumer products like cookware, food packaging, and stain repellants. PFAS manufacturing and processing facilities, airports, and military installations that use firefighting foams are some of the main sources of PFAS. PFAS may be released into the air, soil, and water, including sources of drinking water. PFOA and PFOS are the most studied PFAS chemicals and have been voluntarily phased out by industry, though they are still persistent in the environment. There are many other PFAS, including GenX chemicals and PFBS in use throughout our economy.

Gen X Chemicals: GenX is a trade name for a technology that is used to make high-performance fluoropolymers (e.g., some non-stick coatings) without the use of perfluorooctanoic acid (PFOA). HFPO dimer acid and its ammonium salt are the major chemicals associated with the GenX technology. GenX chemicals have been found in surface water, groundwater, finished drinking water, rainwater, and air emissions in some areas.

PFBS: PFBS is a replacement chemical for PFOS, a chemical that was voluntarily phased out by its U.S. manufacturers. PFBS has been identified in the environment and consumer products, including surface water, wastewater, drinking water, dust, carpeting and carpet cleaners, floor wax, and food packaging.

How are people exposed to GenX chemicals and PFBS?

People can be potentially exposed to GenX chemicals and PFBS through a number of different pathways, including drinking water, inhaling air, and consuming food wrapped in PFAS containing products. (Note: The Food and Drug Administration is responsible for food packaging.) While EPA is continuing to work with state, tribal, and local partners to gather additional information, the agency believes that exposure to GenX chemicals through the ambient air and drinking water is likely localized or regional in nature. EPA will continue to work collaboratively with partners to increase the amount national data on the occurrence of GenX chemicals in water.

EPA recognizes that humans have the potential to be exposed to PFAS through drinking water and other exposure sources. EPA's draft assessments for GenX chemicals and PFBS focus solely on the potential human health effects associated with oral exposure to each chemical; they do not consider potential cumulative (mixture) effects of GenX chemicals and PFBS, or their possible interactions with other PFAS and/or other chemicals.

What health effects are associated with GenX chemicals and PFBS?

Overall, the available oral toxicity studies show that the liver is sensitive to GenX chemicals, and the kidney and thyroid are sensitive to PFBS. EPA has requested public comment on these complex relationships.

GenX Chemicals: Animal studies have shown health effects in the kidney, blood, immune system, developing fetus, and especially in the liver following oral exposure. The data are suggestive of cancer.

PFBS: Animal studies have shown health effects on the thyroid, reproductive organs and tissues, developing fetus, and kidney following oral exposure. Overall, the thyroid and kidney are particularly sensitive to PFBS. The data are inadequate to evaluate cancer.

What is an EPA toxicity assessment?

As public officials work to protect public health, they first must assess the risks before they can identify a plan to manage them. A toxicity assessment is part of the risk assessment process and is a written summary of the potential health effects associated with a chemical that identifies the levels at which those health effects may occur. Specifically, the draft GenX chemicals and PFBS toxicity assessments cover the first two steps (Step 1. Hazard Identification and Step 2. Dose-Response) of the four-step risk assessment process developed by the National Academy of Sciences. Risk characterization, which is not done in these toxicity assessments, requires additional consideration of exposure. For more details about this process: https://www.epa.gov/risk/conducting-human-health-risk-assessment.

When issued, the toxicity values from the GenX chemicals and PFBS assessments can be combined with specific exposure information (Step 3. Exposure Assessment) to help characterize the potential public health risks associated with exposure to these chemicals (Step 4. Risk Characterization).

EPA will continue to work with our state, tribal, and local partners to provide technical assistance, including information about appropriate regulations and statutes, as they begin considering the public comments and revised toxicity values along with relevant exposure scenarios.
After the full risk assessment process is completed, public officials can work to identify how to manage the identified risk. It is through this process that the supporting science, as well as statutory and other legal considerations, risk management options, public health considerations, cost/benefit considerations, economic factors, social factors, and other considerations are weighed.

What are the draft reference doses for PFBS and GenX chemicals?

As part of EPA's draft toxicity assessment, the agency has developed draft oral reference doses (RfDs) for GenX chemicals and PFBS. A reference dose is an estimate of the amount of a chemical a person can ingest daily over a lifetime (chronic RfD) or less (subchronic RfD) that is unlikely to lead to adverse health effects. EPA will continue to work with state, tribal, and local partners to provide technical assistance should they wish to use the final values with relevant exposure scenarios to develop risk assessments to support risk management decisions.

Chemical	Draft Subchronic RfD (mg/kg-day)	Draft Chronic RfD (mg/kg-day)
PFBS*	0.04 (candidate based on thyroid effects)	0.01 (candidate based on thyroid effects)
	0.1 (candidate based on kidney effects)	0.01 (candidate based on kidney effects)
GenX Chemicals	0.0002	0.00008
*The EPA developed candidate values based on thyroid and kidney effects and is requesting public review and comment on these candidate values for PFBS.		

To learn more about EPA's toxicity values, please visit: https://www.epa.gov/iris/basic-information-about-integrated-risk-information-system

How does the toxicity of PFBS and GenX chemicals compare to PFOA and PFOS?

The draft RfD for PFBS suggests it is less toxic than GenX chemicals, PFOA, and PFOS. The draft RfD for GenX chemicals suggests that they are less toxic than PFOA and PFOS. However, these draft values may change in response to public comment.

Toxicity is only one piece of information that public officials consider when determining whether there is a risk to public health. Other factors, such as exposure, must also be considered.

Chemical	Chronic RfD (mg/kg-day)
PFBS	0.01*
GENX chemicals	0.00008*
PFOA	0.00002
PFOS	0.00002
	*indicates draft value

How might GenX chemicals or PFBS impact my drinking water?

EPA recommends that you contact your local water utility to learn more about your drinking water and to see whether they have provided any specific recommendations for your community. GenX chemicals and PFBS typically come from manufacturing processes and industrial releases. These compounds can migrate in the environment and impact the quality of surface water and groundwater which may be used as sources of drinking water. If you own a private well, EPA recommends learning more about how to protect and maintain your well for all contaminants of concern. For information on private wells visit: www.epa.gov/privatewells

Should states, tribes, or local communities use EPA's draft toxicity values now?

No, these draft toxicity values are not final and may change following the public comment period. EPA is issuing the draft toxicity assessments for PFBS and GenX chemicals for public comment to give interested stakeholders an opportunity to provide input to the agency. The public will have 60 days after publication in the Federal Register to provide input. At the end of the comment period, EPA will consider the input and issue the assessments.

Does EPA plan to issue a regulation for these chemicals?

Not at this time. EPA is making the draft toxicity assessments available to provide states, tribes and local governments with the tools they need to better understand PFBS and GenX chemicals. Once the assessments are issued, state, tribal, and local partners can use this information to help inform whether local actions are needed to protect public health.

How can I comment on the draft toxicity assessments?

Public input plays a critical role in EPA's process for issuing the draft toxicity assessments. The agency is accepting public comments for 60 days following publication in the Federal Register. Submit your comments, identified by Docket ID No. EPA-HQ-OW-2018-0614, to the public docket at: http://www.regulations.gov. To view the draft toxicity assessments and other related information on GenX chemicals and PFBS, visit www.epa.gov/pfas/genx-pfbs.

Engagement and Peer Review

EPA is following through on its commitment to work in close collaboration with our federal and state partners to develop draft toxicity assessments for GenX chemicals and PFBS. EPA has engaged with federal, tribal, and state partners throughout the development of the draft toxicity assessments, including both before and after an external peer review.

Federal and tribal partners included:

- U.S. Department of Defense (DoD)
- U.S. Department of Energy (DOE)
- U.S. Geological Survey (USGS)
- U.S. Department of Health and Human Services (HHS), including the Food and Drug Administration (FDA), Agency for Toxic Substances and Disease Registry (ATSDR), the National Institute of Environmental Health Science (NIEHS) including the National Toxicology Program, and other offices
- U.S. Department of Veterans Affairs (VA)
- National Aeronautics and Space Administration (NASA)
- National Toxics Tribal Council (NTTC)
- Office of Management and Budget (OMB)

EPA also engaged extensively with the Association of State Drinking Water Administrators (ASDWA) and five state partners recommended by the Environmental Council of the States (ECOS): Colorado, Michigan, Minnesota, New Hampshire, and Ohio.

Review Process

EPA discussed the assessment process, available data, and methods to be used to derive toxicity values (in this case, reference doses) for GenX chemicals and PFBS with the federal, tribal, and state partners. After external peer review, EPA also discussed the comments received and how EPA planned to address those comments with the partners.

EPA held additional detailed discussions with North Carolina's Department of Health and Human Services (DHHS) and Department of Environmental Quality (DEQ) to continue the agency's efforts to provide technical assistance as the state develops its own technical assessment for GenX chemicals for its Science Advisory Board (SAB) review. EPA also presented its available data and approaches to North Carolina's SAB.

Independent Peer Review

In June and July 2018, EPA conducted a contractor-led independent, external peer review of the initial draft assessments. The peer review panel included five experts in the areas of general risk assessment; benchmark dose modeling; liver, kidney, hematological, thyroid, immune, and reproductive and developmental toxicity; PFAS chemistry; and toxicity. In general, the peer reviews were favorable and

in each case the reviewers provided important feedback that is reflected in the draft toxicity assessments.

Next Steps

Following closure of the 60-day public comment period, the EPA will consider the comments, revise the draft documents and consider the need for additional peer review, as appropriate, and then publish the toxicity assessments.

EXHIBIT C-67

Technical Fact Sheet: Draft Toxicity Assessments for GenX Chemicals and PFBS

EPA is releasing draft toxicity assessments for public comment for hexafluoropropylene oxide (HFPO) dimer acid and its ammonium salt (referred to as GenX chemicals) and perfluorobutane sulfonic acid (PFBS) and its potassium compound salt, perfluorobutane sulfonate (K+PFBS) based on the Agency's analysis of the best available science on the health effects of these chemicals. The values included in these draft assessments are not final and may change following the public comment period. The EPA is issuing the draft toxicity assessments for PFBS and GenX chemicals for public comment to give the public an opportunity to provide input to the Agency. When final, these toxicity assessments can be used by EPA and other federal agencies, and state, tribal, and local communities, along with specific exposure and other relevant information, to determine, under appropriate regulations and statutes, if and when it is necessary to take action to address potential risk associated with human exposure to PFAS. Following closure of the 60-day public comment period, the EPA will consider the comments, revise the draft documents, as appropriate, and publish final toxicity assessments.

Background on GenX Chemicals and PFBS

GenX chemicals and PFBS are man-made, fluorinated organic chemicals that are part of a larger group referred to as per- and polyfluoroalkyl substances (PFAS). PFAS are used in many applications because of their unique physical properties such as resistance to high and low temperatures, resistance to degradation, and nonstick characteristics.

GenX is a trade name for a processing aid technology used to make high-performance fluoropolymers without the use of perfluorooctanoic acid (PFOA). HFPO dimer acid and its ammonium salt are the major chemicals associated with GenX processing aid technology. GenX chemicals have been found in surface water, groundwater, finished drinking water, rainwater, and air emissions in some areas.

PFBS is a four-carbon PFAS that was developed as a replacement for perfluorooctane sulfonic acid (PFOS), a chemical that was voluntarily phased out by its U.S. manufacturers. PFBS has been identified in the environment and consumer products, including surface water, wastewater, drinking water, dust, carpeting and carpet cleaners, floor wax, and food packaging.

PFBS and GenX chemicals are persistent in the environment and mobile in groundwater and surface water.

EPA's Draft Toxicity Assessments

In the risk assessment/risk management paradigm, a toxicity assessment is on the risk assessment side of the paradigm. The GenX chemicals and PFBS toxicity assessments cover the first two steps (Step 1. Hazard Identification and Step 2. Dose-Response) of the four-step risk assessment process, described by the National Academy of Science in 1983 as "the characterization of the potential adverse health effects of human exposures to environmental hazards." Characterizing risk, which is not done in these toxicity assessments, would require additional consideration of exposure. For further details about risk assessment see: https://www.epa.gov/risk/conducting-human-health-risk-assessment.

EPA's draft toxicity assessments for GenX chemicals and PFBS include the first two steps of the risk assessment paradigm described above, including developing oral references doses (RfDs). An RfD is a type of toxicity value specifically for non-cancer effects associated with the oral (ingested) route of exposure. A reference dose is an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily oral exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime. It can be derived from a no-observed-adverse-effect level (NOAEL), lowest-observed-adverse-effect level (LOAEL), or benchmark dose, with uncertainty factors generally applied to reflect limitations of the data used. The higher the RfD, the higher the chemical dose needed to elicit potential adverse health effects.

EPA followed the general guidelines for risk assessment set by the National Research Council (1983) and characterized in a variety of EPA risk assessment guidance and recommendations[1] in identifying the hazards and determining the points of departure (PODs) for the derivation of the RfDs for these chemicals. Consistent with the recommendations presented in EPA Guidelines, EPA considered and applied uncertainty factors to the POD to address, where applicable, intraspecies variability, interspecies variability, extrapolating from the LOAELs to the NOAELs, deficiencies in the database, and extrapolation of study data from a subchronic to a chronic exposure duration.

When final, the toxicity values from the GenX chemicals and PFBS assessments can be combined with specific exposure information (Step 3. Exposure Assessment) by government and private entities to help characterize (Step 4. Risk Characterization) public health risks of these chemicals. Thus, once the GenX chemicals and PFBS assessments are final, EPA will work with our state, tribal, and local partners to provide technical assistance, including information about appropriate regulations and statutes, as they begin considering the final values in relevant exposure scenarios. It is the risk management part of the risk assessment/risk management paradigm where the supporting science, as well as statutory and legal considerations, risk management options, public health considerations, cost/benefit considerations, economic factors, social factors, and other considerations are weighed.

The draft toxicity assessments underwent independent, external expert peer review in June and July 2018. EPA considered the peer reviewers' comments and revised the draft assessments accordingly. External peer review comments and EPA's responses can be viewed at: www.epa.gov/pfas.

[1] www.epa.gov/risk/risk-assessment-guidelines#tab-1

GenX Chemicals: Health Effects Summary

Oral animal (rat, mouse) toxicity studies for HFPO dimer acid and its ammonium salt conducted according to test guidelines (i.e., Organisation for Economic Co-operation and Development (OECD) and EPA Office of Pollution Prevention and Toxics (OPPT)) were available for acute, short-term, subchronic, and chronic durations of exposure. Additionally, oral animal studies reported liver toxicity (increased relative liver weight, hepatocellular hypertrophy, and single cell necrosis), kidney toxicity (increased relative kidney weight), immune effects (antibody suppression), developmental effects (increased early deliveries and delays in genital development), and cancer (liver and pancreatic tumors) at doses ranging from 0.5 mg/kg-day to 1000 mg/kg-day. Overall, the toxicity studies available demonstrate that the liver is particularly sensitive to HFPO dimer acid- and HFPO dimer acid ammonium salt-induced toxicity. Currently, there are not enough data to determine the mode of action GenX chemicals are operating under to illicit these effects in animals.

GenX Chemicals: Reference Dose

The critical study chosen for determining the subchronic and chronic RfDs for HFPO dimer acid and its ammonium salt is the oral reproductive/developmental toxicity study in mice and the critical effect is in the liver (single cell necrosis in males) (DuPont-18405-1037, 2010). While other effects were observed (kidney toxicity, immunological effects, developmental effects, and cancer), effects on liver were observed consistently across all studies which supported selection of this endpoint and critical study. Liver effects were observed in both male and female mice and rats at varying durations of exposures and doses. Because liver effects such as increases in liver weight and hypertrophy can be associated with activation of cellular peroxisome proliferator-activated receptor (PPAR)-α, a process unique to rodents, the EPA assessed the relevance of the liver effects to humans using established criteria (Hall et al., 2012). Based on these criteria, only those doses associated with effects classified as adverse in humans (e.g., histologic or clinical pathology indicative of liver toxicity such as changes in liver enzyme concentrations in the serum, necrosis, inflammation, and degeneration) were used for the point of departure (POD) quantification.

Using EPA's *Benchmark Dose Technical Guidance Document* (2012), benchmark dose modeling was used to empirically model the dose-response relationship in the range of observed data. Consistent with the EPA's Benchmark Dose Technical Guidance (USEPA, 2012), the BMD and the BMDL were estimated using a BMR of 10% extra risk for dichotomous data to facilitate a consistent basis of comparison across endpoints, studies, and assessments. The best fitting model was the Multistage 2 model based on adequate p values (>0.1), the BMDLs are sufficiently close, and the Multistage 2 model had the lowest Akaike information criterion (AIC). Additionally, EPA's *Recommended Use of Body Weight$^{3/4}$ as the Default Method in Derivation of the Oral Reference Dose* (2011) was used to allometrically scale a toxicologically equivalent dose of orally administered agents from adult laboratory animals to adult humans. The use of allometric scaling addresses some aspects of cross-species extrapolation of toxicokinetic and toxicodynamic processes (i.e., interspecies UF). The resulting $BMDL_{10}$ POD human equivalent dose (HED) is 0.023 mg/kg-day. UFs applied include a 10 for intraspecies variability (UF_H), 1 because the POD is a BMDL (UF_L), 3 for interspecies differences (UF_A), 1 for extrapolation from a subchronic to a chronic duration (UF_S), and 3 for database deficiencies (UF_D) in immune effect studies and additional developmental studies, to yield a subchronic RfD of 0.0002 mg/kg-day (Table 1). For the chronic RfD, the same UFs were applied with the addition of a UF_S of 3, which resulted in a chronic RfD of 0.00008 mg/kg-day (Table 1).

Table 1. Summary of Draft Reference Doses for GenX Chemicals

	Critical Study	Critical Effect	POD HED*	Total UF	Draft RfD
Draft Subchronic RfD	Reproductive/ developmental toxicity study; DuPont-18405-1037 (2010)	Single cell necrosis in the liver	$BMDL_{10}$ = 0.023 mg/kg-day	UF_H-10 UF_A-3 UF_L-1 UF_S-1 UF_D-3 Total UF-100	**0.0002 mg/kg-day**
Draft Chronic RfD	Reproductive/ developmental toxicity study; DuPont-18405-1037 (2010)	Single cell necrosis in the liver	$BMDL_{10}$ = 0.023 mg/kg-day	UF_H-10 UF_A-3 UF_L-1 UF_S-3 UF_D-3 Total UF-300	**0.00008 mg/kg-day**

** Allometric scaling adjustment according to EPA guidance using default body weight ¾ scaling*

PFBS: Health Effects Summary

High and medium confidence animal (rat and mouse) toxicity studies from oral exposure to PFBS and its potassium salt were available for acute, short-term, subchronic, and gestational exposure durations, as well as a two-generation reproductive toxicity study. A group of low and medium confidence observational human studies of PFBS exposure and health effects were identified, but their ability to inform conclusions was limited. Health outcomes evaluated across available studies included effects on the thyroid, reproductive organs and tissues, developing offspring, liver, lipids and lipoproteins, immune system, and kidneys following oral exposure to PFBS. Overall, from the identified targets of PFBS toxicity, the thyroid and kidney are particularly sensitive targets of PFBS-induced toxicity.

PFBS: Candidate Reference Doses

Candidate subchronic and chronic RfDs were derived for both thyroid and kidney effects associated with PFBS. For thyroid effects, the critical study chosen for determining the candidate subchronic and chronic RfDs for PFBS and its potassium salt is the oral gestational exposure study in mice (Feng et al., 2017) and the critical effect is on the thyroid (decreased serum total thyroxine) in offspring. Using EPA's Benchmark Dose Technical Guidance Document (2012), benchmark dose modeling was used to empirically model the dose-response relationship in the range of observed data. Additionally, EPA's Recommended Use of Body Weight$^{3/4}$ as the Default Method in Derivation of the Oral Reference Dose (2011) was used to allometrically scale a toxicologically equivalent dose of orally administered agents from animals to humans. The use of allometric scaling addresses some aspects of cross-species extrapolation of toxicokinetic and toxicodynamic processes (i.e., interspecies UF). The resulting point of departure (POD) human equivalent dose (HED) is 4.2 mg/kg-day. Uncertainty factors applied include a 10 for intraspecies variability (UF_H), 3 for interspecies differences (UF_A), 1 because the POD is a BMDL (UF_L), 1 because the POD comes from a developmental study (UF_S), and 3 for database deficiencies (UF_D), including the lack of developmental neurotoxicity and immune effect studies, to yield a candidate subchronic RfD of 0.04 mg/kg-day (Table 2). In the derivation of the chronic RfD, in addition to the

uncertainty factors above, the UF_D was increased to 10 to account for the lack of chronic duration studies, to yield a candidate chronic RfD of 0.01 mg/kg-day (Table 2).

For kidney effects, the critical study chosen for determining the candidate subchronic and chronic RfDs for PFBS and its potassium salt is the two-generation reproductive toxicity study in rats (Lieder et al., 2009b) and the critical effect is kidney histopathology, specifically papillary epithelial tubular/ductal hyperplasia in adult female rats. Using EPA's Benchmark Dose Technical Guidance Document (2012), benchmark dose modeling was used to empirically model the dose-response relationship in the range of observed data. Additionally, EPA's Recommended Use of Body Weight$^{3/4}$ as the Default Method in Derivation of the Oral Reference Dose (2011) was used to allometrically scale a toxicologically equivalent dose of orally administered agents from animals to humans. The use of allometric scaling addresses some aspects of cross-species extrapolation of toxicokinetic and toxicodynamic processes (i.e., interspecies UF). The resulting point of departure (POD) human equivalent dose (HED) is 11.5 mg/kg-day. Uncertainty factors applied include a 10 for intraspecies variability (UF_H), 3 for interspecies differences (UF_A), 1 because the POD is a BMDL (UF_L), 1 because the POD comes from a study of subchronic duration (UF_S), and 3 for database deficiencies (UF_D), including the lack of developmental neurotoxicity and immune effect studies, to yield a candidate subchronic RfD of 0.1 mg/kg-day (Table 2). In the derivation of the chronic RfD, in addition to the uncertainty factors above, the UF_S was increased to 10 to account for the lack of chronic duration studies, to yield a candidate chronic RfD of 0.01 mg/kg-day (Table 2).

In light of the consistent observation of the thyroid effects across life stages and the greater dose response sensitivity, relative to the kidney effects, EPA is proposing to base the overall subchronic and chronic RfDs on the thyroid effects. See the Federal Register Notice announcing the availability of the draft assessment for PFBS and requesting public review and comment on this proposal in addition to the approaches and conclusions in the PFBS assessment.

Table 2. Summary of Draft Reference Doses for PFBS

	Critical Study	**Critical Effect**	**POD (HED)***	**Total UF**	**Draft Candidate RfD**
Draft Candidate Subchronic RfD (thyroid effects)	Gestational exposure study (GD1-20); Feng et al. (2017)	Decreased serum total T4 in newborn (PND1) mice	$BMDL_{20}$ = 4.2 mg/kg-day	UF_H-10 UF_A-3 UF_L-1 UF_S-1 UF_D-3 Total UF-100	**0.04 mg/kg-day**
Draft Candidate Subchronic RfD (kidney effects)	Two-generation reproductive study; Lieder et al. (2009b)	Kidney histopathology – papillary epithelial tubular/ductal hyperplasia in adult female rats	$BMDL_{10}$ = 11.5 mg/kg-day	UF_H-10 UF_A-3 UF_L-1 UF_S-1 UF_D-3 Total UF-100	**0.1 mg/kg-day**
Draft Candidate Chronic RfD (thyroid effects)	Gestational exposure study (GD1-20); Feng et al. (2017)	Decreased serum total T4 in newborn (PND1) mice	$BMDL_{20}$ = 4.2 mg/kg-day	UF_H-10 UF_A-3 UF_L-1 UF_S-1 UF_D-10 Total UF-300	**0.01 mg/kg-day**
Draft Candidate Chronic RfD (kidney effects)	Two-generation reproductive study; Lieder et al. (2009b)	Kidney histopathology – papillary epithelial tubular/ductal hyperplasia in adult female rats	$BMDL_{10}$ = 11.5 mg/kg-day	UF_H-10 UF_A-3 UF_L-1 UF_S-10 UF_D-3 Total UF-1000	**0.01 mg/kg-day**

** Allometric scaling adjustment according to EPA guidance using default body weight ¾ scaling*

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Chronic Toxicity Comparison

EPA previously published final health effects support documents for two other PFAS: PFOA and PFOS. Based on these EPA assessments, the draft chronic RfD for GenX chemicals is within one order of magnitude (4x) higher than the chronic RfDs for PFOA and PFOS (Table 3). The draft chronic RfDs for PFBS are approximately three orders of magnitude (~1000x) higher than the chronic RfDs for these other PFAS (Table 3). Therefore, based on currently available animal toxicity data, it appears that GenX chemicals are slightly less toxic and PFBS is much less toxic than PFOA and PFOS.

Table 3. Comparison of Chronic Toxicity for PFAS With EPA Health Effects Assessments

Chemical [Citation]	**EPA Chronic RfD [mg/kg-day]**	**Critical Effect (Study)**
GenX Chemicals [EPA 2018a (*PUBLIC REVIEW DRAFT*)]	0.00008	Single cell necrosis in the liver (DuPont 18405-1037, 2010)
PFBS [EPA 2018b (*PUBLIC REVIEW DRAFT*)] – Candidate RfD for thyroid effects	0.01	Decreased serum T4 in newborn mice (Feng et al., 2017)
PFBS [EPA 2018b (*PUBLIC REVIEW DRAFT*)] – Candidate RfD for kidney effects	0.01	Increased incidence of kidney papillary epithelial tubular/ductal hyperplasia in the rats (Lieder et al., 2009b)
PFOA [EPA 2016a (FINAL)]	0.00002	Skeletal effects and accelerated puberty in males (Lau et al., 2006)
PFOS [EPA 2016b (FINAL)]	0.00002	Decreased pup weight in rats (Luebker et al., 2005)

Applications for Risk Assessment and Risk Management

Following publication of final health effects assessments, these RfDs will provide information on health effects and may be used to inform health-based national standards, clean-up levels at local sites, and non-regulatory advisory levels. RfDs can be applied in a variety of exposure scenarios to characterize potential risk from chemical exposure and develop health protective levels for chemicals in water, soil, and other media. For example, RfDs can be combined with exposure information to develop regulatory standards (e.g., Maximum Contaminant Levels) or non-regulatory guideline values (e.g., health advisories) for drinking water under the Safe Drinking Water Act (SDWA), and human health water quality criteria for permitting discharges into ambient waters under the Clean Water Act (CWA). RfDs are also used in risk assessments under the Comprehensive Environmental Response, Compensation and Liability Act (CERCLA), also known as Superfund; under the CWA for pollutants in biosolids; and under the Resource Conservation and Recovery Act (RCRA) to develop cleanup levels for contaminated soil and groundwater. The levels developed for these risk management tools may vary due to the type of exposure being evaluated. As such, the RfD is not meant to be the standard itself, but the starting point for risk managers to develop those standards.

Once final, the EPA will work with our state, tribal, and local partners to provide technical assistance as they begin considering the final values in relevant exposure scenarios. It is the risk management part of the risk assessment/risk management paradigm where the supporting science, as well as statutory and legal considerations, risk management options, public health considerations, cost/benefit considerations, economic factors, social factors, and other considerations are weighed.

How to Learn More and Provide Comments

To view the draft toxicity assessments and other related information on GenX chemicals and PFBS, visit www.epa.gov/pfas.

Submit your comments, identified by Docket ID No. EPA-HQ-OW-2018-0614, to the public docket at: http://www.regulations.gov. Follow the online instructions for submitting comments. Once submitted, comments cannot be edited or withdrawn. For additional submission methods, the full EPA public comment policy, information about confidential business information or multimedia submissions, and general guidance on making effective comments, please visit http://www2.epa.gov/dockets/commenting-epa-dockets.

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EXHIBIT C-68

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Environmental Health

RESEARCH Open Access

Prenatal exposure to perfluoroalkyl and polyfluoroalkyl substances and the risk of hypertensive disorders of pregnancy

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Abstract

Background: Perfluoroalkyl and polyfluoroalkyl substances (PFAS) have been reported to disrupt endocrine system and reproduction. However, epidemiological evidence on the association between PFAS and preeclampsia is inconsistent. We aimed to investigate the association between prenatal PFAS exposure and hypertensive disorders of pregnancy (HDP) in humans.

Methods: PFAS were measured by liquid chromatography system coupled with tandem mass spectrometry in 687 umbilical cord plasma samples collected between 2011 and 2012 in Shanghai, China. Information on HDP including gestational hypertension and preeclampsia was abstracted from medical records. Multiple logistic regression was used to examine the association of each PFAS with gestational hypertension, preeclampsia, and overall HDP in separate models. Elastic net regression with logit link was used to identify independent associations between exposures and outcomes. Logistic regression was used to obtain the unpenalized estimates of the selected PFAS components for the associations with outcomes, adjusting for age, education level, pre-pregnancy BMI, parity, and mutual adjustment of selected PFAS.

Results: The risk of gestational hypertension and preeclampsia was 3.3% and 2.8% in our subjects, respectively. Perfluorobutane sulfonate (PFBS), perfluorohexane sulfonate (PFHxS), perfluoroundecanoic acid (PFUA) were associated with preeclampsia based on elastic net penalty regression. In the fully adjusted statistical model, women with a higher level of standardized ln-transformed PFBS had an increased odds of preeclampsia [adjusted odds ratio (AOR): 1.81, 95% confidence interval (CI): 1.03–3.17], and overall HDP (AOR: 1.64, 95% CI: 1.09–2.47).

Conclusions: Prenatal exposure to PFBS was positively associated with the risk of preeclampsia and overall HDP.

Keywords: PFAS, Cord blood, Gestational hypertension, Preeclampsia

Introduction

Hypertensive disorders of pregnancy (HDP) are among the most common complications of pregnancy. A national survey involving 112,386 pregnant women from 38 secondary and tertiary hospitals in China in 2011 reported that HDP occurred in 5.2% of pregnancies [1], which is slightly lower than that reported by other international studies [2–4]. HDP are often classified into four categories: 1) chronic hypertension, 2) gestational hypertension, 3) preeclampsia-eclampsia, 4) preeclampsia superimposed on chronic hypertension. Preeclampsia/eclampsia is a more severe form of this disorder and is a major cause of perinatal and maternal morbidity worldwide [5]. It contributes to nearly 10% of stillbirths and 15% of preterm births [6]. It is generally believed that incomplete remodeling of the uterine arteries and insufficient placental perfusion greatly contribute to both gestational hypertension and preeclampsia [7]. However, gestational hypertension seems to be more associated with maternal characteristics than placenta-related factors. For example, it has been reported that women with gestational hypertension are more likely to have higher

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body mass index than women with preeclampsia [8]. Even though the etiology and pathogenesis of HDP have not been fully elucidated, maternal exposure to environmental pollutants has been considered as an important risk factor of HDP [3].

Perfluoroalkyl and polyfluoroalkyl substances (PFAS) are a large group of manufactured compounds widely used in both industrial and consumer products [9]. Humans are exposed to PFAS through various pathways, including food, water, air, indoor dust and soil [10–13]. Most frequently studied PFAS have a long half-life of 3–5 years in human body [14]. Our previous study found that eight common PFAS were detected in more than 90% of umbilical cord blood samples [15]. Among them, perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA) had the highest levels.

Epidemiological studies have provided inconsistent results on the association between PFAS and preeclampsia. Two studies focusing on women who lived in areas with a high PFOA level but with background PFOS level found that PFOA and PFOS were significantly positively associated with preeclampsia with an odds ratio (OR) of 1.1–1.2 across the upper three quintiles for PFOA and OR of 1.13 in relation to a shift from 25th to 75th percentile for PFOS [16, 17], while another study focusing on women with background level of PFAS exposure found that PFOA, PFOS and perfluoroheptane sulfonate (PFHpS) were not associated with preeclampsia, but perfluoroundecanoic acid (PFUA) had an inverse significant association with preeclampsia [HR (95% CI): 0.78 (0.66, 0.92) for per ln-unit] [18]. The inconsistent results may be due to different exposure levels between these two areas. PFAS level in the C8-Health Project took into account the historical PFAS exposure while the study based on Norwegian women did not do so, resulting potential lower estimates of PFAS level due to phase out of PFOS and PFOA in North America and Europe since 2000. The Chinese women probably have a higher level of PFAS exposure than the background exposure level in Norwegian due to continuous production and use of PFAS in China and relocation of PFAS production to China [19]. In addition, a short-chain PFAS, perfluorobutane sulfonate (PFBS) which has been increasingly produced to replace PFOS, has been detected in cord blood samples in the Chinese women [15, 20]. Experimental studies have reported adverse effects of PFBS on the immune and endocrine functions [21, 22]. Therefore, it is possible that these adverse effects may also interfere with the remodeling of uterine spiral arteries, the pivotal feature of normal placentation, and contribute to the development of preeclampsia and gestational hypertension [23, 24]. But whether PFBS is associated with preeclampsia and gestational hypertension is still unknown. Therefore, this study aimed to examine the association of PFAS exposure with preeclampsia and gestational hypertension in the Chinese population.

Methods

Study design and participants

The present analysis is a cross-sectional study. From 2011 and 2012, 687 women who had a singleton pregnancy and came for delivery at two large hospitals in Shanghai were recruited. A face-to-face interview was conducted by trained nurses to collect information on maternal age, education level, pre-pregnancy weight, and height. Information on parity and pregnancy-related complications was abstracted from medical records. Cord blood samples were collected shortly after birth. Among the 687 subjects, 13 subjects were excluded because of missing information on maternal age, education level, pre-pregnancy BMI, and parity, resulting in 674 valid subjects. A written consent was obtained from each woman. This study was approved by the Ethics Committees of all involved research institutions and hospitals.

Outcomes

As noted above, information on chronic hypertension before pregnancy, gestational hypertension, and preeclampsia was obtained from medical records. Gestational hypertension was defined as new onset of hypertension (systolic blood pressure ≥ 140 mmHg or diastolic blood pressure ≥ 90 mmHg) after 20 gestational weeks. Preeclampsia was defined as new onset of hypertension (systolic blood pressure ≥ 140 mmHg or diastolic blood pressure ≥ 90 mmHg) after 20 gestational weeks accompanied by proteinuria (a urine dipstick of at least +). According to the International Society for the Study of Hypertension in Pregnancy, hypertensive disorders of pregnancy (HDP) included gestational hypertension, preeclampsia, chronic hypertension (essential or secondary), or pre-eclampsia superimposed on chronic hypertension [25]. Chronic hypertension before pregnancy and preeclampsia superimposed on chronic hypertension were excluded in the current analysis because our study focused on the association between PFAS and pregnancy-related hypertension.

Blood sampling and exposure assessments

Umbilical cord blood samples were collected at delivery and centrifuged at 4000 rpm for 10 min immediately after collection, with plasma separated and stored at −80 °C until shipping on dry ice to the laboratory for analysis. Detailed method of measuring PFAS has been described elsewhere [15]. A total of 100 μL was used to measure PFAS for each plasma sample. This method has been cross-validated with a research lab at the Aarhus University in Denmark. PFOSA and PFHpA were not included as they are detected in < 30% samples, PFOS,

PFNA, and PFHxS were detected in all the samples, while PFBS, PFOA, PFDA, perfluoroundecanoic acid (PFUA), and perfluorododecanoic acid (PFDoA) had a detection rate above 90%. Those that were not detected were assigned half of the limit of detection (LOD; 0.00 45 ng/mL for PFBS, 0.045 ng/mL for PFOA, 0.01 ng/mL for PFDA and PFUA, 0.09 ng/mL for PFOS, 0.03 ng/mL for PFHpA, 0.02 ng/mL for PFNA and PFHxS, 0.12 ng/mL for PFOSA and 0.025 ng/ml for PFDoA). The interassay coefficients of variation (CV) was between 1.7 and 8.4%, and the intra-assay CV was between 0.8 and 8.5% [15].

Statistical analysis

Considering the multiple correlations between these PFAS, elastic net regression was used to select the exposures that are associated with outcome, while simultaneously accounting for other PFAS exposures. Elastic net regression is a penalized regularization method combined with the properties of ridge and least absolute shrinkage and selection operator (LASSO) penalty [26]. It is well known that in the case of multi-collinearity among examined exposure, ridge penalty shrinks the coefficients of correlated exposures towards each other while LASSO selects one of them and discard the others, avoiding the unstable estimates of correlated exposures in ordinary regression approaches [27]. However, ridge regression retains all predictor variables and cannot produce a parsimonious model, and LASSO regression selects a subset of predictors and owns a poor prediction performance relative to ridge regression if there are high correlations between predictors [26]. By combining ridge and LASSO penalty, elastic net regression does variable selection and continuous shrinkage, and selects groups of correlated exposures as a whole. As such, it has been widely used as a multi-pollutant model that support the identification of the dominant pollutants that are associated with outcome while dealing with multi-collinearity in environmental epidemiological studies [28, 29]. For the elastic net regression with logit link, the turning parameters of ridge and LASSO penalty were selected via 10-fold cross-validation (CV) with the selection criteria of minimum misclassification error. The process was repeated 100 times. After the variable selection, the associations of PFAS with gestational hypertension, preeclampsia, and overall HDP were reassessed by separate multiple logistic regression models.

For regression models, a set of covariates that were selected based on a directed acyclic graph were set as confounders (Additional file 1: Figure S1), including maternal age, educational level, parity and pre-pregnancy BMI (calculated as weight in kilograms divided by height in meters squared). Because only a small proportion of mothers smoke before pregnancy ($N = 10$, proportion = 1.5%) and during pregnancy ($N = 3$, proportion = 0.4%) in our subjects, smoking is unlikely to confound the association assessed in this study and was not included as a confounder. These covariates were included in the elastic net regression without penalization. Before regression analysis, PFAS concentrations were first ln-transformed to mitigate the effects of their right-skewed distribution and then were centered and standardized with one-standard deviation based on ln-transformed scale. Additionally, PFAS concentration was analyzed as both a continuous variable (scaled ln-transformed) and categorical variables in tertiles (T1/T2/T3) with the lowest tertile (T1) being the reference group. *P*-values for linear trend of odds ratio for hypertensive disorders of pregnancy in relation to different level of each PFAS concentration was obtained by treating the categorical PFAS variables as continuous variables in the regression model. Preeclampsia might affect kidney function and alter PFAS concentration, which may be most prominent for PFAS with a short half-life, such as PFBS. To improve the assessment of whether such as effect might have occurred, a sensitivity analysis was conducted for PFBS with further adjustment of birthweight and gestational age. Results were given in Additional file 2: Table S1. Statistical analyses were conducted using RStudio version 1.1.453 (2009–2018 RStudio, Inc) and fit elastic net regression using the glmnet package.

Results

The risk of gestational hypertension, preeclampsia and overall HDP was 3.3, 2.8, and 6.1%, respectively, in our study. Table 1 shows that the mean age of these women was 29.3 years, with an average pre-pregnancy BMI of 21.3 kg/m^2. More than 90% of them were nulliparous, and nearly 90% had college education. Women with HDP were a little older [Mean (SD) of HDP vs Normotensive women: 30.6 (4.5) vs 29.2 (3.8), $P = 0.02$], and had a higher pre-pregnancy BMI than normotensive wo men [Mean (SD) of HDP vs Normotensive women: 23.1 (3.8) vs 21.2 (3.1), $P < 0.001$].

A total of 8 PFAS components were quantifiable in more than 70% of blood samples (Table 2). PFOA had the highest median concentration (6.98 ng/ml), followed by PFOS (2.38 ng/ml), PFNA (0.64 ng/ml), PFUA (0.40 ng/ml), PFDA (0.36 ng/ml), PFHxS (0.16 ng/ml), PFDoA (0.094 ng/ml) and PFBS (0.047 ng/ml). Figure 1 shows that the Pearson correlation coefficients between the eight PFAS components ranged from 0.01 to 0.95.

Table 3 shows odds ratio and corresponding 95% CI of the association between each PFAS and hypertensive disorders of pregnancy after adjusting for potential confoun ders.

Table 4 shows that after adjusting for potential confounders, in the multiple-exposure elastic net regressions, PFBS, PFHxS and PFDoA were selected (beta

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Table 1 Basic characteristics of the subjects by hypertensive disorders of pregnancy (HDP) ($n = 686$)

	Total ($n = 686$) Mean (SD) or N (%)	HDP ($n = 42$)	Normotensive ($n = 644$)	#P values
Age (years)	29.3 (3.8)	30.6 (4.5)	29.2 (3.8)	0.02
Pre-pregnancy BMI (Kg/m^2)	21.3 (3.2)	23.1 (3.8)	21.2 (3.1)	< 0.001
Parity				
Nulliparous	626 (91.5)	39 (92.9)	587 (91.4)	0.4
Parous	58 (8.5)	3 (7.1)	55 (8.6)	
Education level				
Less than college	95 (13.9)	4 (9.5)	91 (14.1)	
[a]College degree	529 (77.2)	33 (78.6)	496 (77.1)	0.6
Postgraduate degree	61 (8.9)	5 (11.9)	56 (8.7)	

[#]P values were from two-tailed Student's t tests for continuous variables, and Chi-square tests for categorical variables between HDP and normotensive women
[a]College degree: post-secondary education with 3 or 4 years education in college or university

coefficient from elastic net regression [β_{EN}]~ = 0) for the overall HDP, while PFBS, PFHxS and PFUA were selected for preeclampsia, none of the PFAS components was selected for gestational hypertension.

Table 5 shows that in the unpenalized logistic regression models with adjustment of potential confounders, one unit increase in standardized PFBS concentration was associated with a higher risk of HDP [Adjusted odds ratio (AOR): 1.64, 95% CI: 1.09–2.47], and preeclampsia (AOR: 1.81, 95% CI: 1.03–3.17). PFHxS (AOR: 0.79, 95% CI: 0.55–1.13) and PFDoA (AOR: 0.76, 95% CI: 0.55–1.04) were non-significantly and negatively associated with HDP. PFHxS (AOR: 0.82, 95% CI: 0.49–1.37) and PFUA (AOR: 0.82, 95% CI: 0.53–1.27) were non-significantly and negatively associated with preeclampsia.

Additional file 2: Table S1 shows the results of the association between PFAS and hypertensive disorders of pregnancy with further adjustment of gestational age and birth weight in addition to those adjusted in the model presented in Table 5. Similar results were found.

We also explored the joint effect of the PFAS on hypertensive disorders in pregnancy using the structural equation model (Additional file 3: Figure S2). The odds ratio and corresponding 95% confidence interval (95% CI) of PFAS were 0.99 (0.97, 1.02), 0.98 (0.95, 1.01) and 0.98 (0.94, 1.01) for preeclampsia, gestational hypertension, and hypertensive disorders of pregnancy, respectively.

Discussion

Our study used elastic net regression models to select a subset of PFAS components most strongly related to HDP and found that PFBS exposure during pregnancy was significantly positively associated with HDP and preeclampsia. However, this study found that preeclampsia was not associated with PFOA and PFOS, which were different from that found in the US-based C8 Health Project which reported both PFOA and PFOS were significantly positively associated with preeclampsia [16, 17]. This study is also different from another study from Norway which found that PFUA had an inverse association with preeclampsia, while PFOS and PFHpS had no association with preeclampsia [18]. The differences between these studies may be due to different measure of PFAS exposure and statistical analysis methods, or different diagnosis methods of preeclampsia.

Table 2 Plasma concentrations of 8 perfluoroalkyl and polyfluoroalkyl substances in our sample

Perfluoroalkyl substances	Abbreviated Name	% > LOD	Plasma concentration (ng/ml) by percentile		
			25th	50th	75th
Perfluorooctanoic acid	PFOA	99.9	4.95	6.98	9.54
Perfluorooctane sulfonate	PFOS	100	1.81	2.38	3.23
Perfluorononanoic acid	PFNA	100	0.50	0.64	0.83
Perfluoroundecanoic acid	PFUA	99.9	0.29	0.40	0.53
Perfluorodecanoic acid	PFDA	99.1	0.23	0.36	0.54
Perfluorohexane sulfonate	PFHxS	100	0.132	0.16	0.20
Perfluorododecanoic acid	PFDoA	90.4	0.069	0.094	0.13
Perfluorobutane sulfonate	PFBS	97.2	0.037	0.047	0.061

LOD (ng/ml): PFOSA (0.12), PFHpA (0.03), PFOS (0.09), PFNA (0.02), PFHxS (0.02)

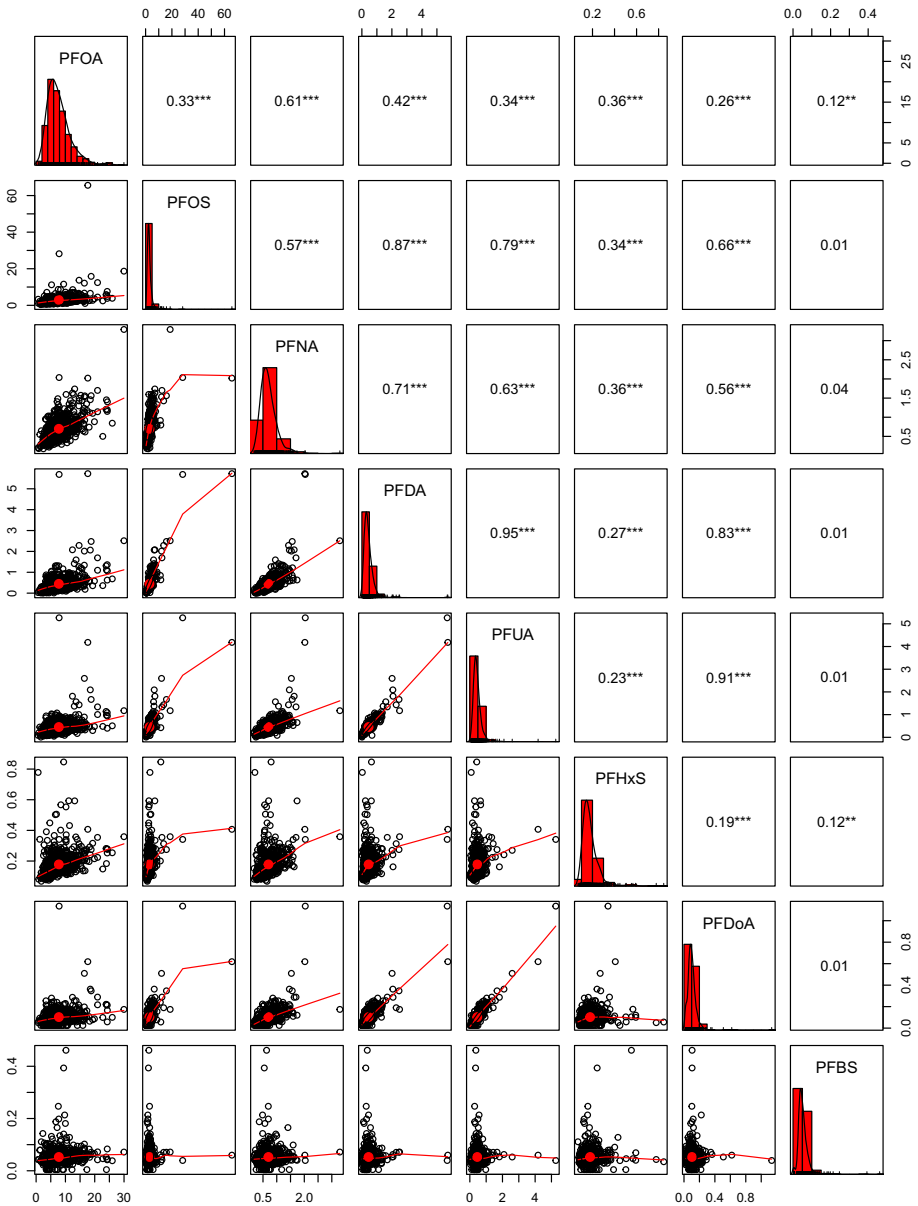

Fig. 1 Correlation coefficients among the PFAS. $^{**}P < 0.01$, $^{***} < P < 0.001$. Note: Non-straight lines mean that the relation between two PFAS may be non-linear

PFAS was measured in cord blood samples in this study. However, maternal blood samples during mid-pregnancy was used in the study focusing on Norwegian women [18], PFOA was indirectly estimated using environmental, exposure, and pharmacokinetic modeling for each participant [17], and blood samples collected up to 5 years after pregnancy were used to estimate PFAS concentration [16]. Although PFAS in cord blood was used to estimate maternal level in this study, it was reported that PFAS concentration in the cord plasma ranged between 30 to 79% of maternal concentration for PFOA, PFOS, PFHxS, PFUA, PFNA [30], and nearly 100% for PFBS [31].

PFOA in this study was more than two times that reported in the study of Norwegian women [18], which was consistent with another study in Shanghai reporting that maternal serum concentration of PFOA was much higher (mean = 11.6 ng/mL) than that in Norwegian counterparts (1.5 ng/mL) [32]. However, PFOA in this study was slightly lower than that reported in the C8-Health Project, which is consistent with previous two studies in Shanghai [13, 17, 32]. Without adjusting for other PFAS, our study is consistent with the C8-Health Project in that PFOA was positively but non-significantly associated with preeclampsia.

Table 3 Odds ratios for hypertensive disorders of pregnancy associated with cord blood concentrations of perfluoroalkyl and polyfluoroalkyl substances

	Hypertensive disorders of pregnancy [a]OR (95% CI)	Preeclampsia	Gestational hypertension
PFOA			
T1	1	1	1
T2	0.85 (0.38–1.88)	2.23 (0.67–7.44)	0.33 (0.10–1.11)
T3	0.96 (0.44–2.10)	1.41 (0.38–5.14)	0.77 (0.30–2.01)
[b]Standardized	1.02 (0.73–1.44)	1.12 (0.68–1.84)	0.95 (0.61–1.48)
PFOS			
T1	1	1	1
T2	0.55 (0.24–1.25)	0.59 (0.19–1.87)	0.54 (0.17–1.66)
T3	0.82 (0.39–1.72)	0.70 (0.23–2.08)	0.95 (0.36–2.49)
[b]Standardized	0.85 (0.62–1.17)	0.83 (0.52–1.32)	0.87 (0.57–1.34)
PFUA			
T1	1	1	1
T2	1.29 (0.62–2.68)	0.89 (0.31–2.52)	1.75 (0.65–4.70)
T3	0.64 (0.27–1.52)	0.51 (0.15–1.76)	0.81 (0.25–2.66)
[b]Standardized	0.82 (0.61–1.10)	0.82 (0.55–1.23)	0.84 (0.58–1.22)
PFDA			
T1	1	1	1
T2	1.23 (0.58–2.59)	1.16 (0.38–3.53)	1.26 (0.48–3.31)
T3	0.78 (0.34–1.80)	1.00 (0.31–3.19)	0.63 (0.20–2.00)
[b]Standardized	0.85 (0.63–1.14)	0.94 (0.60–1.46)	0.79 (0.55–1.15)
PFDoA			
T1	1	1	1
T2	0.92 (0.44–1.93)	1.25 (0.43–3.59)	0.71 (0.26–1.91)
T3	0.53 (0.23–1.22)	0.60 (0.17–2.14)	0.50 (0.17–1.50)
[b]Standardized	0.74 (0.55–1.00)	0.83 (0.54–1.29)	0.70 (0.47–1.03)
PFNA			
T1	1	1	1
T2	0.38 (0.15–0.94)	0.28 (0.06–1.37)	0.47 (0.16–1.41)
T3	0.87 (0.43–1.79)	1.40 (0.51–3.83)	0.56 (0.20–1.41)
[b]Standardized	0.86 (0.62–1.19)	1.10 (0.33–3.71)	0.74 (0.48–1.15)
PFHxS			
T1	1	1	1
T2	0.93 (0.44–1.99)	1.10 (0.36–3.38)	0.83 (0.31–2.22)
T3	0.59 (0.26–1.34)	0.80 (0.25–2.60)	0.48 (0.16–1.43)
[b]Standardized	0.77 (0.54–1.09)	0.81 (0.49–1.33)	0.75 (0.47–1.19)
PFBS			
T1	1	1	1
T2	1.0 (0.40–2.47)	2.08 (0.51–8.50)	0.55 (0.16–1.92)
T3	2.21 (1.00–4.88)	3.41 (0.91–12.7)	1.57 (0.59–4.17)
[b]Standardized	1.53 (1.04–2.25)	1.69 (0.98–2.90)	1.36 (0.82–2.25)

Abbreviations: *T1* tertile 1, *T2* tertile 2, *T3* tertile 3

[a]Adjusting for age, education, pre-pregnancy BMI, and parity

[b]Standardized: PFAS concentration was subtracted by mean and then divided by its standard deviation

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Table 4 Multiple-exposure elastic net penalized regression models (β_{EN}) for hypertensive disorders of pregnancy

PFAS	Hypertensive disorders of pregnancy	Preeclampsia	Gestational hypertension
PFOA	0		0
PFOS	0		0
PFUA	0	−0.10	0
PFDA	0		0
PFDoA	−0.20		0
PFNA	0		0
PFHxS	−0.13	−0.06	0
PFBS	0.32	0.34	0

Regression coefficients (β_{EN}) represent the change in log-odds per increment in standardized ln-transformed PFAS

However, the study focusing on the Norwegian women did not find such an association. Both this study and the study on the Norwegian women used preeclampsia cases validated by medical records, while self-reported preeclampsia was used in the C8-Health Project [16–18]. Usage of validated preeclampsia case could largely exclude the possibility of misclassification of outcomes.

PFOS in this study was much lower than reported in the other studies [16, 18]. This is partly due to that the efficiency of placental transfer for PFOS is only about

Table 5 Logistic regression models for the selected exposures and hypertensive disorders of pregnancy/preeclampsia

PFAS	Hypertensive disorders of pregnancy	Preeclampsia
	[a]AOR (95% CI)	
PFBS		
[b]Standardized	1.64 (1.09–2.47)	1.81 (1.03–3.17)
T1 (≤0.0398)	1	1
T2 (0.0399–0.0554)	0.89 (0.39–2.44)	2.09 (0.51–8.53)
T3 (0.0556–0.4612)	2.26 (1.02–5.02)	3.51 (0.94–13.2)
P value for linear trend	0.03	0.05
PFHxS		
[b]Standardized	0.79 (0.55–1.13)	0.82 (0.49–1.37)
T1 (≤0.11402)	1	1
T2 (0.1403–0.1831)	0.94 (0.43–2.03)	1.14 (0.36–3.58)
T3 (0.1834–0.8465)	0.61 (0.26–1.41)	0.92 (0.27–3.11)
P value for linear trend	0.79	0.88
PFDoA		
[b]Standardized	0.76 (0.55–1.04)	
T1 (≤0.0775)	1	NA
T2 (0.0776–0.1118)	0.89 (0.42–1.88)	
T3 (0.112–1.1357)	0.54 (0.23–1.29)	
P value for linear trend	0.77	NA
PFUA		
[b]Standardized	NA	0.82 (0.53–1.27)
T1 (≤0.3276)		1
T2 (0.3277–0.4808)		0.83 (0.29–2.41)
T3 (0.4819–5.2653)		0.49 (0.13–1.75)
P value for linear trend	NA	0.28

Variance inflation variance (VIF) for exposures ranged from 1.01 to 1.1

[a]Adjusting for age, education, pre-pregnancy BMI, parity and mutual adjustment of PFAS including in the corresponding model

Abbreviations: *T1* tertile 1, *T2* tertile 2, *T3* tertile 3

[b]Standardized: PFAS concentration was subtracted by mean and then divided by its standard deviation

30% [30]. Previous studies have shown that PFOS level of maternal blood samples was similar in Shanghai, Norway, and US [13, 16, 18]. PFOS in cord blood was highly correlated with that in maternal blood (spearman correlation coefficient = 0.74) [30], so the cord blood PFOS level is well representative of the level in maternal blood. Nevertheless, neither PFOA nor PFOS was selected to be associated with preeclampsia in the elastic net regression model which accounts for the correlation between each PFAS.

It has been reported that PFAS in maternal and cord blood samples could be highly correlated, with correlation coefficients ranging from 0.52 to 0.95 [33, 34]. Low correlation might have attenuated the association between PFAS and HDP. The pathogenesis of preeclampsia has not been fully understood, but shallow placentation and endothelial dysfunction may play a key role [35]. It is believed that defective placentation, with shallow trophoblast invasion into the maternal decidual and spiral arteries in early pregnancy, is the starting point in the pathogenesis of preeclampsia [35]. Consequently, placental hypoperfusion and ischemia may lead to changes in the cytokine production and secretion. The imbalance in these cytokines may lead to maternal endothelial dysfunction and subsequently affect cardiovascular system and cause high blood pressure [35].

Despite that PFBS is assumed to be a safe substitute of PFOS, several studies have shown that PFBS has endocrine disruption effects [21, 36], toxicity in human placental trophoblast cells [21] and neuronotypic cells [37], immunotoxicity [22, 38], and transcriptional effects [39]. Some of these toxicities coincide with the pathophysiology of preeclampsia [35], but no previous epidemiological studies have explored the association between prenatal PFBS exposure and HDP.

PFBS may have adverse effects on the immune and endocrine function in human cells, at a concentration level that did not cause cytotoxicity. It has been reported that PFBS interfered with inflammatory cytokines and NF-κB activation, affecting the fine-tuning of pro- and anti-inflammatory microenvironment of the uteroplacental site, and contributing to the dysfunction in the trophoblast activities and vascular endothelial cells function [22–24, 40–42]. The endocrine disrupting effects of PFBS on placental cells have also been demonstrated. Gorrochategui et al. found that PFBS may suppress aromatase activity directly in human placental choriocarcinoma cell line [21], disrupting the regulation of the estrogen levels, which is essential for maintaining healthy pregnancy.

Our study has several limitations. Firstly, PFAS were measured in cord blood rather than in maternal blood. One may question the temporality of the association. One would argue that since preeclampsia occurred before birth, the observed association lacks temporality and, therefore, may not be causal. Indeed, it is possible that preeclampsia might affect liver and kidney functions, leading to less secretion of PFBS and more accumulation in the body, i.e., a reverse causation. However, the sensitivity analysis which further adjusted for birth weight and gestational age produced similar results, indicating that birth weight and gestational age were not associated with PFBS level. Therefore, at least the duration of maternal transfer of PFBS was not affected. On the other hand, maternal-fetal transfer of PFAS has been reported in both human studies and animal studies [20, 30, 43–45]. Our own recent research found that the median ratio of PFBS concentration in cord serum to maternal serum was nearly 1 among 369 paired samples of maternal blood and cord blood [31]. The extremely high transmission through the placenta might be due to the short carbon-chain of PFBS [30]. Thus, the cord blood level of PFBS can represent maternal blood level well. However, PFBS level in late pregnancy may not necessarily represent that in early pregnancy. Previous studies have reported that PFOA and PFOS decreased across pregnancy due to increased glomerular filtration rate (GFR) and subsequent increased elimination speed by urine [43, 46–48]. Therefore, PFAS level in multiple time points across pregnancy is desired to better reflect the exposure level. Additionally, the magnitude of the association assessed in this study may be biased if the association between PFAS and hypertensive disorders of pregnancy is not linear. Secondly, the relatively small sample size in this study provided limited statistical power and resulted in wide 95% CIs. However, the direction and magnitude of the associations in this study are unlikely to be substantially biased.

Conclusion

Plasma PFBS in cord blood was positively associated with preeclampsia in a dose-response pattern. Further large-scale prospective studies in human and animal experiments are warranted to examine the relationship between PFBS exposure and preeclampsia, and to elucidate the underlying biological mechanisms.

Additional files

Additional file 1: Figure S1. The directed acyclic graph of the association between each PFAS and hypertensive disorders of pregnancy. (PNG 48 kb)

Additional file 2: Table S1. Logistic regression models for the selected exposures and hypertensive disorders of pregnancy/preeclampsia. (DOCX 15 kb)

Additional file 3: Figure S2. Structural equation model including a joint latent PFAS concentration. The latent PFAS concentration is manifested by the observed PFOA, PFOS, PFHxS, PFNA, PFDoA, PFDA, PFUA, and

PFBS. "Confounders" are age, education level, parity, and pre-pregnancy BMI. (PNG 29 kb)

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Availability of data and materials
The datasets analyzed in this study are available from the corresponding author zhangjun@xinhuamed.com.cn on reasonable request.

Authors' contributions
RH and JZ designed the study. RH analyzed the data, and drafted the manuscript. QC, LZ, and LC collected the data. QC, LC and SZ performed experiments. KL significantly contributed to the data analysis. LF and JZ extensively edited the manuscript. All authors provided critical comments and input in the paper. JZ takes the overall responsibility for the study. All authors have reviewed the final version of the manuscript and agreed to the submission.

Ethics approval and consent to participate
The ethics approval was obtained from the Xinhua Hospital and International Peace Maternity and Infant Health affiliated to the Shanghai Jiao Tong University School of Medicine. This study was conducted in accordance with the principles of the Helsinki Declaration.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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Effect of GenX on P-Glycoprotein, Breast Cancer Resistance Protein, and Multidrug Resistance–Associated Protein 2 at the Blood–Brain Barrier

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Background: Ammonium 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)propanoic acid (GenX) is a replacement for perfluorooctanoic acid in the production of fluoropolymers used in a variety of consumer products. GenX alters fetal development and antibody production and elicits toxic responses in the livers and kidneys of rodents. The GenX effect on the blood–brain barrier (BBB) is unknown. The BBB protects the brain from xenobiotic neurotoxicants and harmful endogenous metabolites.

Objectives: We aimed to investigate the effects of GenX on the transport activity and expression of P-glycoprotein (P-gp), breast cancer resistance protein (BCRP), and multidrug resistance–associated protein 2 (MRP2) at the BBB.

Methods: Transporter activities were measured in isolated rat brain capillaries by a confocal microscopy–based method. ATPase (enzymatic hydrolysis of adenosine triphosphate to inorganic phosphate) levels were measured *in vitro*. Western blotting determined P-gp and BCRP protein levels. Cell survival after GenX exposure was determined for two human cell lines.

Results: Nanomolar levels of GenX inhibited P-gp and BCRP but not MRP2 transport activities in male and female rat brain capillaries. P-gp transport activity returned to control levels after GenX removal. GenX did not reduce P-gp- or BCRP-associated ATPase activity in an *in vitro* transport assay system. Reductions of P-gp but not BCRP transport activity were blocked by a peroxisome proliferator–activated receptor γ (PPARγ) antagonist. GenX reduced P-gp and BCRP transport activity in human cells.

Conclusion: In rats, GenX at 0.1–100 nM rapidly (in 1–2 h) inhibited P-gp and BCRP transport activities at the BBB through different mechanisms. PPARγ was required for the GenX effects on P-gp but not BCRP transport activity. https://doi.org/10.1289/EHP5884

Introduction

Ammonium 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy) propanoate (GenX) (CAS no. 62037-80-3) is a chemical precursor used in the production of polytetrafluoroethylene (Teflon) and as a replacement for perfluorooctanoic acid (Wang et al. 2013). The production volume of GenX in the European Union is estimated to be 10–100 tons per year; however, the worldwide production volume is unknown (Beekman et al. 2016). Although GenX is unlikely to pose a significant aquatic hazard, its bioactivity and persistence in environmental media are problematic (Hoke et al. 2016). GenX is a contaminant in rivers of the Netherlands, Germany, and China (Heydebreck et al. 2015). In June 2017, GenX was detected in the Cape Fear River in eastern North Carolina, United States (Sun et al. 2016). Further surveys nearby and downstream from a PFAS manufacturing facility detected GenX in air, private well water, and some local food products including oysters and honey (Pritchett et al. 2019; Clabby 2018). Most importantly, GenX was detected in finished drinking water. Since GenX has the potential to disrupt biological signaling pathways known to regulate ABC [adenosine triphosphate (ATP) binding cassette] transporters (Conley et al. 2019; Miller and Cannon 2014; More et al. 2017; Zhang et al. 2014), we investigated its effects on three transporters at the BBB: ABCB1, [P-glycoprotein (P-gp)], ABCG2 [breast cancer resistance protein (BCRP)], and ABCC2 [multidrug resistance–associated protein 2 (MRP2)]. We hypothesized that disruption of signaling pathways by GenX would change basal levels of transporter expression and/or activity. ABC transporters are primary active transporters that derive their energy from the hydrolysis of ATP to adenosine diphosphate (ADP) + inorganic phosphate (Pi). Within biological barriers, they function to restrict the access of toxic drugs, endobiotics, and xenobiotics to sensitive cells, tissues, and organs (Locher 2016). In tumor cells, their overexpression leads to multidrug resistance and presents major obstacles in cancer therapies (Bockor et al. 2017; Robey et al. 2018). In most human tissues, P-gp, BCRP, and ABCC2/MRP2 are ubiquitously expressed at low levels; however, in the biological barriers, they are highly expressed and function to protect the brain, retina, testes, and the developing fetus (Nagy et al. 2016). Their localization and expression levels in liver and kidney also serve to eliminate harmful agents from the body (Leslie et al. 2005). Specifically, in the canalicular membrane of liver, they transport conjugates and harmful metabolites into bile. In the proximal tubules of the kidney, they aid in excretory transport of substrates into urine. They are also expressed in the enterocytes of the intestine, where they function to limit the absorption of harmful substrates into the body (Murakami and Takano 2008). The substrates of P-gp, BCRP, and MRP2 are chemically diverse and include chemotherapeutics, lipids, steroids, bilirubin, bile acids, platelet-activating factor, dietary flavonoids, and conjugated endogenous and xenobiotic metabolites (Kim 2002).

Signaling pathways dynamically regulate the activity and expression of P-gp, BCRP, and MRP2 at the BBB (Miller and Cannon 2014). To examine the effects of GenX on these transporters, we exposed rat brain capillaries *ex vivo* to low nanomolar concentrations of GenX. We also dosed rats *in vivo* by oral gavage with 30, 300, or 3,000 pmol/kg GenX and measured transport activity *ex vivo* using a steady-state confocal microscopy–based assay. Lastly, we expanded our study to humans by measuring the effect of GenX

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on the survival of two human cell lines exposed to increasing concentrations of cytotoxic substrates of P-gp and BCRP.

Materials and Methods

Materials

Crystalline GenX (molecular weight of 347.084 g/mol, >97% purity] was purchased from SynQuest Labs. P-gp fluorescent substrate [N-ε-(4-Nitrobenzofurazan-7-yl)-D-Lys8] cyclosporine A (NBD-CSA) was custom synthesized by R. Wenger (Sandoz) (Schramm et al. 1995). MRP2 fluorescent substrate Texas Red®, Sigma-Aldrich (sulforhodamine MRP2 inhibitor MK-571, corn oil, and ß-actin mouse monoclonal antibody A1978 were purchased from Sigma-Aldrich. P-gp inhibitor PSC-833 and BCRP inhibitor KO-134 were purchased from Tocris Bioscience. Adriamycin was the gift of the Drug Synthesis and Chemistry Branch, Developmental Therapeutic Program of the National Cancer Institute (NCI), National Institutes of Health (NIH). Mitoxantrone hydrochloride was purchased from Sigma-Aldrich. Both Adriamycin and mitoxantrone were dissolved in double-distilled water (10 mg/mL) and stored at −80°C. P-gp rabbit monoclonal antibody ab170904 was purchased from Abcam. Secondary antibodies, Alexa Fluor® 647 Goat anti-Mouse IgG and Alexa Fluor® 647 Goat anti-Rabbit IgG, were purchased from Thermo Fisher Scientific. Western blotting lysis buffer CelLytic™ MT Mammalian Tissue Lysis/Extraction Reagent with complete mini protease inhibitor was purchased from Sigma-Aldrich. The BCRP substrate BODIPY™ FL prazosin and the Western blotting materials, including 10-well Invitrogen NuPAGE 4-12% Bis-Tris Gels NP0321 and polyvinylidene difluoride (PVDF) Western blot membranes, were obtained from Invitrogen (Thermo Fisher Scientific).

Animals

Male and female Hsd:Sprague-Dawley® (SD®) rats (age 12–15 wk) were purchased from Envigo. Animals were housed in the NIEHS Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC)–approved animal care facility [∼49% humidity, ∼72°F room temperature, 12-h light/dark cycle, polycarbonate shoebox cages (Tecniplast), and Sani-Chip® bedding (PJ Murphy Forest Products)] for at least 1 wk prior to use and allowed access to food (NIH #31) and tap water *ad libitum* consumption. Animals were euthanized by CO_2 inhalation followed by decapitation. All animal protocols were approved by the Animal Care and Use Committee at the NIEHS according to the guidelines from the NIH. All data are reported in compliance with the Animal Research Reporting *In Vivo* Experiments (ARRIVE) guidelines.

Capillary Isolation and ex Vivo *ABC Transport Assay*

To measure *ex vivo* transport activity of P-gp and BCRP at the blood–brain barrier (BBB), capillaries from male and female rats were isolated as previously reported (Chan and Cannon 2017). Briefly, the brains from four to six male or female rats were harvested following euthanasia and placed in assay buffer [1 × phosphate-buffered saline (PBS), pH 7.4, supplemented with 900 mg/mL of glucose and 110 mg/mL of sodium pyruvate] on ice. Cortical gray matter was isolated by discarding white matter, meninges, midbrain, choroid plexus, and olfactory lobes using dissecting forceps and a stereomicroscope. The remaining cortical gray matter enriched for capillaries was minced with a razor blade, suspended in 15 mL isolation buffer, and homogenized by 40 up-and-down strokes using a Thomas (size C) mechanical tissue grinder (Thomas Scientific; catalog no. 3431E55) matched with a size C serrated pestle (Thomas Scientific; catalog no. 3431F25; clearance: 150 to 230 μM) rotating at 50 rpm. A final homogenization was performed by 10 up-and-down strokes using a 15-mL KONTES® Dounce tissue grinder (pestle, size B; catalog no. 885300-0015; clearance: 165 to 889 μm; VWR). The resulting homogenate was suspended in an equal volume of ice-cold 30% Ficoll PM400 dissolved in assay buffer to achieve a final Ficoll PM 400 content of 15% wt/vol and centrifuged for 20 min at 5,800 × *g* at 4°C using an RC-5B Centrifuge (Sorvall) with a SS-34 rotor. Following centrifugation, supernatants were removed, and pelleted capillaries were resuspended in assay buffer containing 1% bovine serum albumin (BSA) (Sigma-Aldrich) and captured by passage through a 30-μM filter [pluriStrainer (Pluriselect), 43-50030-03]. BSA from Sigma was removed from isolated capillaries by three sequential centrifugations at 900 × *g* following resuspension in 15 mL isolation buffer (no BSA). After the third centrifugation, the capillaries were resuspended in 0.5 mL assay buffer, and equal volumes were loaded into sterile borosilicate chamber slides purchased from Thermo Scientific (catalog no. 155380). The chamber slides were incubated at room temperature for 15 min to allow capillary settling and binding. Next, the chamber slides were rinsed with 1 mL assay buffer. To measure transporter activity, chamber slide bound capillaries were incubated at 0–4 h in 2 mL of assay buffer containing a fluorescent substrate (2 μM) specific for each transporter (NBD-CSA for P-gp, BODIPY™ FL prazosin for BCRP, and Texas Red® for MRP2). Transporter activity was determined as the mean measurement of steady-state luminal fluorescence for 15–20 individual capillaries per chamber. Luminal fluorescence reaches a steady state in 30 min at room temperature (Chan and Cannon 2017). Nonspecific background fluorescence was determined by measuring the luminal fluorescence of fully inhibited capillaries. All inhibitors were administered as a 30-min pretreatment before fluorescent substrates were added. The inhibitors used were PSC-833 (10 μM, P-gp), KO134 (20 μM, BCRP), and MK-571 (20 μM, MRP2). Specific transport for each transporter was calculated as the difference between total noninhibited transport minus nonspecific inhibited transport. To measure luminal fluorescence, confocal images of capillaries were captured using a Zeiss 710 confocal microscope. Luminal fluorescence was quantified using FIJI/ImageJ (File version, ImageJ 1.52a; Java 1.8.0_112) (NIH) analysis software. The solvent [0.1% vol/vol dimethylsulfoxide (DMSO)] was used as the vehicle control (VC) to match the concentration of the treatment solvents. GenX in 1 × PBS compared to GenX in DMSO 0.1% produced no significant effects on P-gp transport (Figure S1). For all relevant experiments, GenX was dissolved in DMSO immediately prior to use. Negative controls employed inhibitors specific for each transporter. Where relevant, GenX and the peroxisome proliferator–activated receptor γ (PPARγ) antagonist, GW9662 (50 nm) were freshly dissolved in DMSO and used alone or in cotreatments (inhibitor studies) in volumes (0.1% vol/vol) to match VC volumes. When needed, staggered time courses were used to accommodate confocal image acquisitions at 10 min/dose group.

Measuring Transport Activity after GenX Removal

The reversibility assays are a modified design of the *ex vivo* transport assay. Briefly, isolated capillaries from four to six rats were pooled and equally distributed into chamber slides and incubated at room temperature in 2 mL assay media with a VC (DMSO vol/vol 0.1%) and the P-gp- or BCRP-specific fluorescent substrate (2 μM). In our VC group, steady-state transport activity was established by measuring luminal fluorescence after 1–5 h incubation at room temperature. In a second group, GenX was added to achieve a 100-nM final concentration in media containing the fluorescence substrates and incubated for 1 h for P-gp and 2 h in the BCRP transport assays. At 1–2 h, respectively, P-gp and BCRP transport

activities were measured. Following transport measurements, GenX was removed by two washes (equal volumes) with assay media containing fluorescence substrates specific for each transporter. After GenX removal, the samples were incubated in assay media with appropriate substrates. Next, P-gp and BCRP transport activities were measured at 0.5, 1, 2, and 3 h after GenX removal. All transporter activities were determined as the mean measurement of steady-state luminal fluorescence minus background fluorescence for 15–20 individual capillaries per chamber. Capillaries were imaged using a Zeiss 710 confocal microscope. Luminal fluorescence was quantified using FIJI/ImageJ analysis software. All transport experiments, including the reversibility assays, were performed two times to ensure reproducibility.

ATPase Activation Assay

The ATPase assay was used to determine if GenX at concentrations of 0.001, 0.01, 0.1, and 1.0 μM influenced the ATPase activity associated with P-gp and BCRP transport activity in a purified system. The assay is based on the spectrophotometric quantitation of Pi produced from P-gp- or BCRP ATPase-mediated conversion of ATP to ADP + Pi when the substrate was stimulated with 10 μM paclitaxel for P-gp and 10 μM sulfasalazine for BCRP. To accomplish this, 10 μL of membrane vesicles, provided by the assay kit, containing either 2 μg of P-gp or BCRP proteins, were diluted 10-fold in assay buffer and transferred to a well of a 96-well plate in triplicate. The wells contained either *a*) P-gp or BCRP plus substrates (stimulated positive control), *b*) P-gp or BCRP plus substrates with the ATPase inhibitor vanadate (1.25 mM) (ATPase negative control), *c*) P-gp or BCRP minus substrates (negative control), *d*) P-gp or BCRP minus substrates with 1.0 μM GenX, or *e*) P-gp or BCRP plus substrates and GenX in DMSO 0.1% at 0.001, 0.01, 0.1, or 1.0 μM final concentration. The mixtures were preincubated at 37°C for 10 min, and the reaction was started by the addition of 10 μL Mg-ATP (200 mM) and transporter-specific substrates or DMSO 0.1% where appropriate. The plate was incubated at 37°C for 20 min. All reactions were terminated by the addition of 40 μL of 5% sodium dodecyl sulfate. Pi levels are a function of ABC transporter activity, which requires the hydrolysis of ATP to ADP + Pi. To measure Pi, a colorimetric detection solution was prepared by mixing one part of 35 mM ammonium molybdate in 15 mM zinc acetate (pH 5.0) with three parts of 10% ascorbic acid. The final detection solution was mixed by inversion, and 200 μL was added to each sample and incubated for 20 min at 37°C. Assays were read at 650 nm on a Gemini™ spectrometer (Molecular Devices) and graphed as relative units of ATPase activity. The means of triplicate samples were calculated using Prism software (version 7.05; GraphPad). Data are expressed as mean ± standard error (SE).

GenX in Vivo *Dosing Studies*

Male and female rats (five/dose group) received a single oral gavage dose of high-performance liquid chromatography (HPLC)–grade water as a VC or GenX in HPLC-grade water at 30, 300, or 3,000 pmol/kg (10, 100, or 1,000 ng/kg, respectively). All doses were given in a volume of 4 mL/kg. Animals were euthanized at 5 h postdosing. Brains from each dose group were pooled, and capillaries were isolated as previously described above. P-gp and BCRP transport activities were measured *ex vivo* as described above.

GenX Cytotoxicity in Human Cells

The P-gp overexpressing the human ovarian NCI/ADR-RES cell line was obtained from NCI at Frederick Cancer Center (Scudiero et al. 1998). It was selected for its reliable high expression of P-gp. A second line, MX-MCF-7, was selected for its high expression levels of BCRP. This mitoxantrone-resistant MX-MCF-7 BCRP-expressing cell line (Nakagawa et al. 1992) was a generous gift of Dr. E. Schneider, Wadsworth Center, New York State Department of Health. Both low passage lines (<10 passages) were grown in Phenol Red-free RPMI media supplemented with 10% fetal bovine serum and antibiotics and used up to 15–20 passages, after which the cells were discarded and a new cell culture started from fresh frozen stock. Both cell lines were used in cytotoxicity studies that measured cell growth inhibition. First, we determined the cytotoxic effect of GenX (alone) for each line. Cell growth inhibition assays were performed for each line by plating 100,000–125,000 (NCI/ADR-RES for P-gp and MX-MCF-7 for BCRP) cells/well in triplicate. Each was grown for 18 h at 37°C and 5% CO_2 to allow attachment. Next, they were grown in fresh media containing increasing concentrations of GenX (10^{-9} to 10^{-4} M). After 72 h, the cultures were washed three times with 50 mL 1 × PBS to remove nonadherent cells. The remaining adherent cells were harvested by trypsinization and counted in a Beckman Coulter Counter (Beckman).

To determine if GenX (100 nM) affected the toxicity of known cytotoxic substrates for P-gp or BCRP, for each line, we plated 100,000–125,000 (NCI/ADR-RES for P-gp and MX-MCF-7 for BCRP) cells/well in triplicate. Each was grown for 18 h at 37°C and 5% CO_2 to allow attachment. Next, they were grown in fresh media containing 100 nM GenX and the toxic P-gp substrate, Adriamycin (10^{-8} to 10^{-5} M). MX-MCF-7 cells were grown in media for 72 h with or without 100 nM GenX plus the toxic BCRP substrate mitoxantrone (10^{-9} to 10^{-4} M). After 72 h, the cultures were washed three times with 50 mL 1 × PBS to remove nonadherent cells. The remaining adherent cells were harvested by trypsinization and counted in a Beckman Coulter Counter (Beckman).

Gel Electrophoresis and Western Blotting

Isolated capillaries pooled from 6 rats were treated with VC (DMSO 0.1%) or 100 nM GenX at room temperature in 15 mL Falcon tubes (Fisher Scientific; catalog no. 14-959-53A) containing 5 mL assay buffer (1 × PBS, pH 7.4, supplemented with 900 mg/L of glucose and 110 mg/L of sodium pyruvate). After treatment, capillaries were pelleted by centrifugation for 15 min at a centrifugal force of 1,860 × *g* at 4°C and stored at −80°C until use. Membrane-containing protein lysates were isolated by adding 200 μL of lysis buffer (CelLytic™ MT Cell Lysis Reagent; Sigma-Aldrich; catalog no. C3228-500; with Roche Complete Mini protease inhibitor cocktail; catalog no. 4693159001) to each pellet. Capillary pellets were kept on ice and vortexed for 30 s every 10 min for 90 min. In addition, the samples were sonicated at 4°C for 60 s at the 20-, 40-, and 60-min time points. To separate the nuclei from cytoplasm and cellular membranes, the samples were centrifuged at 10,000 × *g* for 30 min. The pellets containing nuclei were discarded, and the supernatants containing cytoplasm and membranes were centrifuged at 100,000 × *g* for 90 min. The liquid cytosolic fraction was removed, and the remaining membrane pellet was dissolved in 50 μL MT cell (Sigma-Aldrich) lysis buffer and stored at −80°C until use. Membrane protein concentration was determined as described by the Coomassie Protein Assay Kit 23200 (Thermo Scientific), a modified version of the Bradford assay (Bradford 1976). BSA protein standards were provided by the kit. Electrophoresis and Western blotting were performed according to the manufacturer's instructions. Briefly, 1 μg of capillary membrane lysates were mixed with 1 × reducing agent and 1 × loading buffer (Invitrogen), loaded into NuPAGE Bis-Tris Gels (4–12%) using the XCell SureLock Mini-Cell Electrophoresis System (Invitrogen), and electrophoresed in MOPS Running Buffer (Invitrogen) for 50 min at a constant 200 V. Following electrophoresis, the resolved proteins were transferred from the gel to a PVDF membrane (Invitrogen) using the XCell II

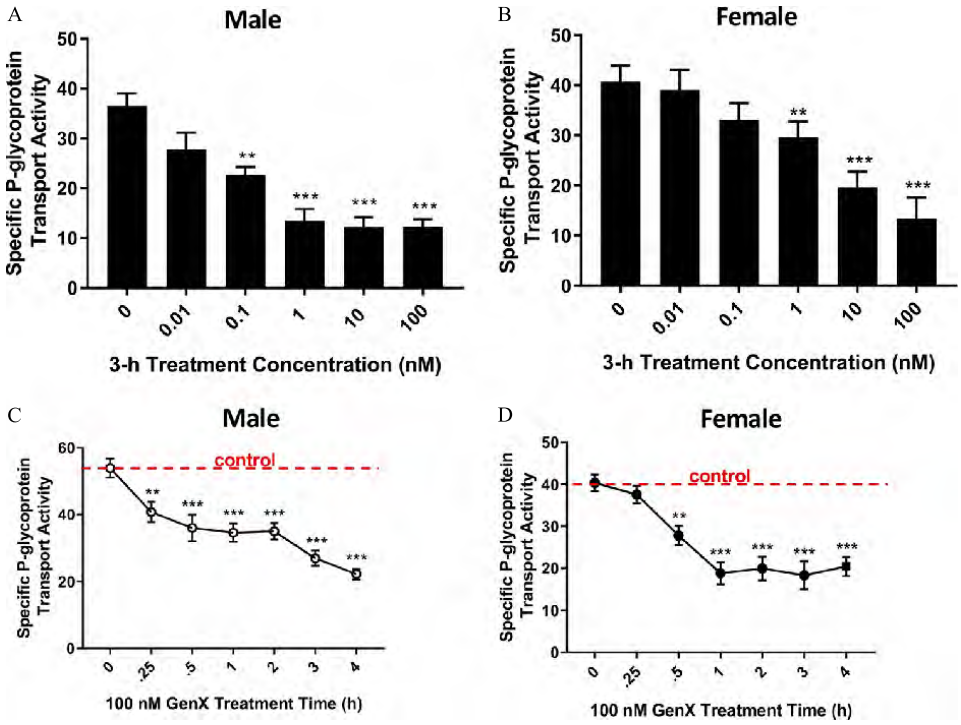

Figure 1. Changes in P-glycoprotein (P-gp) transport activity in brain capillaries from six rats treated with ammonium 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)propanoic acid (GenX). (A) Male and (B) female are graphs of P-glycoprotein (P-gp) transport activity at increasing doses of GenX in brain capillaries of Hsd:Sprague-Dawley® (SD®) rats. (C) Male and (D) female are graphs of the GenX-dependent reductions in P-gp transport activity over time. Dotted horizontal line denotes vehicle control (no GenX) levels. Mean ± standard error (SE) is shown. SE and significance were determined by one-way analysis of variance (ANOVA) and Tukey multiple comparison. Significance is as compared to control unless otherwise specified: $^{**}p < 0.01$; $^{***}p < 0.001$.

Blot Module using Invitrogen™ Bolt™ Transfer Buffer plus 10% (vol/vol) methanol. Electrotransfer was performed at constant current (0.1 up to approximately ~0.4 A) or voltage (10 to 25 V) for 60 min. To visualize the P-gp-, BCRP-, and ß-actin-specific bands, the membrane was incubated at room temperature for 30 min in 50 mL blocking buffer (Intercept® Blocking Buffer, Licor). The membrane was washed in 1 × PBS and hybridized overnight at 4°C in 1 × PBS with 0.1% Tween with 1:200 vol/vol P-gp (Abcam; catalog no. 170904) and BCRP (Abcam; catalog no. 207732) primary antibodies and 1:5,000 vol/vol ß-actin primary antibody (Abcam; catalog no. 8224). To remove excess antibody, the membrane was washed three times with 1 × PBS with 0.1% Tween and incubated at room temperature for 1 h in 1 × PBS with 1:10,000 vol/vol secondary antibodies [Odyssey Goat anti-Mouse IR Dye 800CW or Goat anti-Rat IR dye 680CW (Licor)] for an additional 60 min and washed three times with 1 × PBS with 0.1% Tween to remove excess unbound secondary antibody. Specific protein bands were imaged and quantified for protein fluorescence using FluorChem M (ProteinSimple). For each sample, target band intensities were normalized to β-actin. Western blotting was performed in triplicate from three independent experiments. Means and standard errors of the mean (SEM) were calculated using Prism software (version 7.05; GraphPad) software. Data are expressed as a mean ± SE.

Statistics

Luminal fluorescence and cell survival data were analyzed and graphed using Prism software (version 7.05; GraphPad). Data are expressed as mean ± SE, and significant differences between the control and treated means were determined by one-way analysis of variance (ANOVA) and Tukey multiple comparison. For the *ex vivo* transport assay, significance for each data point was determined by comparing treated to control: $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$.

Results

Transporter Activity in Rat Brain Capillaries Treated with GenX

We determined the rapid effects of GenX exposure on the BBB by measuring GenX-mediated changes in *ex vivo* transport activity of three well-characterized ABC transporters (P-gp, BCRP, and MRP2). To accomplish this, we used an established steady state–based confocal microscopy assay (Chan and Cannon 2017). To determine the effect of increasing concentrations of GenX on the ABC transporters at the BBB, we exposed isolated capillaries from male and female rat brains to 0.01–100 nM GenX for 3 h and measured transport activity. In males, P-gp transport activity was lowered but not significantly ($p = 0.13$) by 0.01 nM GenX exposure. P-gp transport activity was significantly lower in male capillaries exposed to 0.01–100 nM GenX (Figure 1A). In females, P-gp transport activity was unchanged by 0.01–0.1 nM GenX exposures but significantly lowered by 1.0–100 nM GenX (Figure 1B). Next, we treated capillaries with 100 nM GenX for 1–4 h and examined the hourly changes in P-gp transport. We chose 100 nM GenX because it produced the greatest reduction in P-gp transport activity for both sexes. Results shown in Figure 1C,D show that 100 nM GenX significantly lowered P-gp transport activity in 15 and 30 min in male and female rat brain capillaries, respectively.

Next, we examined the effect of GenX on BCRP transport activity. We measured BCRP transport activity after treating male and female brain capillaries with GenX (0.1 nM–1 µM) for 3 h (Figure 2A,B). BCRP transport activity in males was significantly lower in samples treated with 1.0 nM–1 µM GenX. In contrast, female BCRP transport activity was significantly lower in capillaries treated with 0.1 nM–1 µM GenX. We also examined the hourly changes in BCRP transport in capillaries following

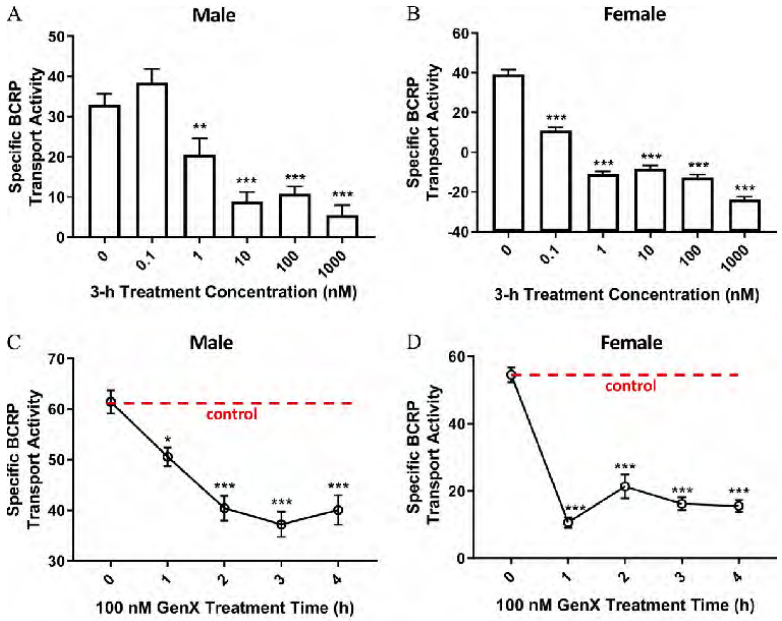

Figure 2. Changes in breast cancer resistance protein (BCRP) transport activity in brain capillaries from six rats treated with ammonium 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)propanoic acid (GenX). (A) Male and (B) female are graphs of BCRP transport activity at increasing doses of GenX in brain capillaries of Hsd:Sprague-Dawley® (SD®) rats. (C) Male and (D) female are graphs of the GenX-dependent reductions in BCRP transport activity over time. Dotted horizontal line denotes vehicle control (no GenX) levels. Mean ± standard error (SE) is shown. SE and significance were determined by one-way analysis of variance (ANOVA) and Tukey multiple comparison. Significance is as compared to control unless otherwise specified: $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.01$.

exposure to 100 nM GenX for 1–4 h (Figure 2C,D). Both male and female BCRP transport activity was significantly lower at 1 h. Of note, the reduction in BCRP transport activity in females was more significant than males at the 1-h time point; furthermore, GenX-dependent transport inhibition persisted throughout the 3-h assay time for both sexes.

Lastly, we determined the GenX effect on MRP2 transport in identically designed experiments. We observed no GenX effect with regard to dose and time on MRP2 transport activity (Figure S2A–D).

Reversibility Assays for P-gp and BCRP Transport

Previous transport studies from our laboratory have shown that chemical perturbation of signal transduction pathways can lead to rapid changes in transport activity independently of protein degradation or expression (Banks et al. 2018; Cannon et al. 2012). In general, these chemical-induced changes in transport activity revert to control levels upon chemical removal. Knowing this, we performed a reversibility assay by exposing male and female rat brain capillaries to GenX (100 nM). Shown in Figure 3, P-gp transport activity in males (Figure 3A) and females (Figure 3B) rapidly reverted to control levels within 1 h after GenX removal. In contrast to P-gp, GenX-mediated decreases in BCRP transport did not revert for either sex (Figure 3C,D).

The Effect of GenX on P-gp and BCRP ATPase Activity in Vitro

To determine if GenX directly inhibited ATP hydrolysis or was a P-gp or BCRP substrate *in vitro*, we used a reconstituted transport assay system containing vesical membranes and purified P-gp or BCRP transport proteins. Using this assay, we compared the control basal levels of substrate-stimulated P-pg or BCRP ATPase activities with and without GenX at 0.001, 0.01, 0.1, and 1.0 μM (Figure 4A,B). GenX at 0.001, 0.01, 0.1, and 1.0 μM did not affect the levels of ATPase activity associated with P-gp or BCRP transport when the substrate was stimulated. Negative controls show the relative ATPase levels were fully inhibited by the ATPase pan-inhibitor, vanadate (1.25 mM). Next, we determined if GenX could stimulate ATP hydrolysis by measuring the effect of 1 μM GenX on each transporter without a substrate (Figure 4A,B). We saw no significant differences in ATPase hydrolysis for P-gp or BCRP, indicating that GenX was not a substrate for either transporter in this system.

P-gp and BCRP Protein Levels following GenX Exposure

To determine if the GenX-mediated decreases in P-gp and BCRP transport activity were associated with decreases in transporter protein levels, we treated male and female rat brain capillaries for 4 h with 100 nM GenX and measured P-gp and BCRP protein levels by Western blotting. Pictured in Figure 5A are representative Western blots showing the P-gp and BCRP protein levels in GenX-treated compared to nontreated male and female rat capillaries. We detected no significant differences in P-gp or BCRP protein levels in GenX (100 nM) or VC-treated capillaries from three independent blots (Figure 5B,C).

Inhibition of PPARγ in GenX-Treated Rat Brain Capillaries

We investigated the role of PPARγ in the GenX-dependent reductions of P-gp and BCRP transport. To accomplish this, isolated capillaries from male and female rats were cotreated for 4 h with GenX (1.0 nM and 100 nM) with and without the PPARγ inhibitor GW9662 (50 nM). Following treatment, P-gp and BCRP transport activities were determined. Shown in Figure 6A, P-gp transport in males was significantly reduced by GenX exposures of 1 nM and 100 nM. However, cotreatments with GW9662 (50 nM) and GenX (1 and 100 nM) blocked the reductions in P-gp transport activity in females (Figure 6B), In females, GenX treatments of 1 nM and 100 nM also lowered P-gp transport activity relative to controls, but in contrast to males, the PPARγ antagonist, GW9662, did not block the 100 nM GenX decreases in P-gp transport. Capillaries cotreated with 100nM GenX and GW9662

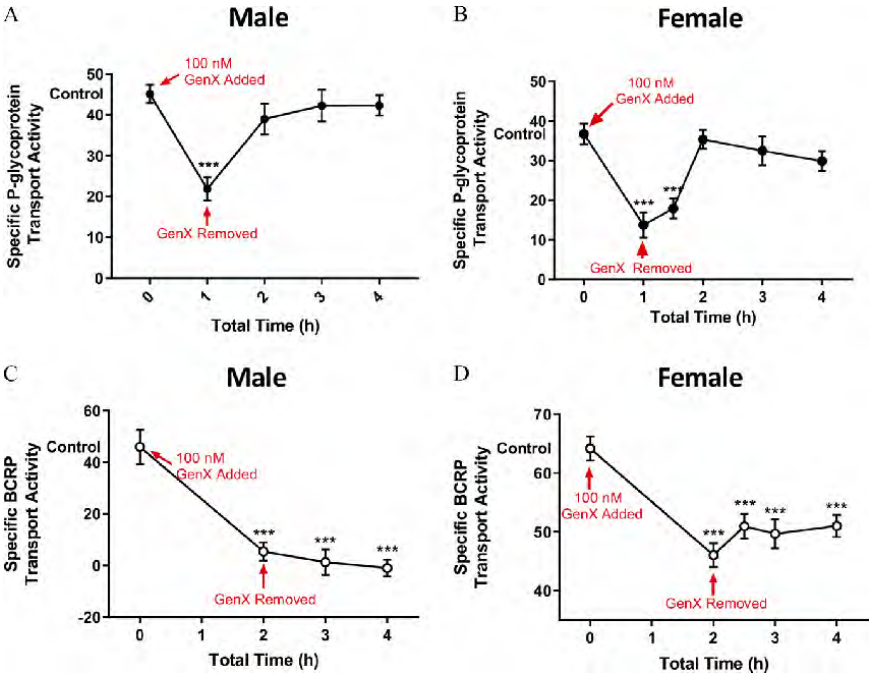

Figure 3. Transport reversibility following ammonium 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)propanoic acid (GenX) removal. (A) Male and (B) female are graphs denoting P-glycoprotein (P-gp) transport activities, and (C) male and (D) female are graphs denoting breast cancer resistance protein (BCRP) transport activities in brain capillaries from six rats before and after GenX (100 nM) removal. Mean ± standard error (SE) is shown. SE and significance were determined by one-way analysis of variance (ANOVA) and Tukey multiple comparison. Significance is as compared to control unless otherwise specified: $^{***}p < 0.001$.

(50 nM) remained significantly lower than their vehicle-treated controls.

In similarly designed experiments, we investigated the involvement of PPARγ on GenX reductions of BCRP transport. Isolated capillaries from male and female rats were cotreated for 4 h with 1.0 nM and 100 nM GenX with or without the PPARγ inhibitor GW9662 (50 nM). Following treatments, BCRP transport activities were measured. Shown in Figure 6C,D, BCRP transport activity in male and female capillaries treated with 1 nM and 100 nM GenX were significantly lower than their vehicle-treated controls. In contrast to our findings with P-gp, cotreating with GenX and GW9662 (50 nM) had no effect on the GenX-mediated reductions in BCRP transport activities in either sex.

In Vivo *Exposures to GenX by Oral Gavage Dosing in Sprague-Dawley Rats*

To validate our *ex vivo* experiments, we dosed male and female SD rats with GenX at 30 pmol/kg, 300 pmol/kg, and 3 nmol/kg by oral gavage and measured P-gp and BCRP transport in isolated rat brain capillaries activity 5 h later. Shown in Figure 7A–D, all GenX dose groups produced significant decreases in P-gp and

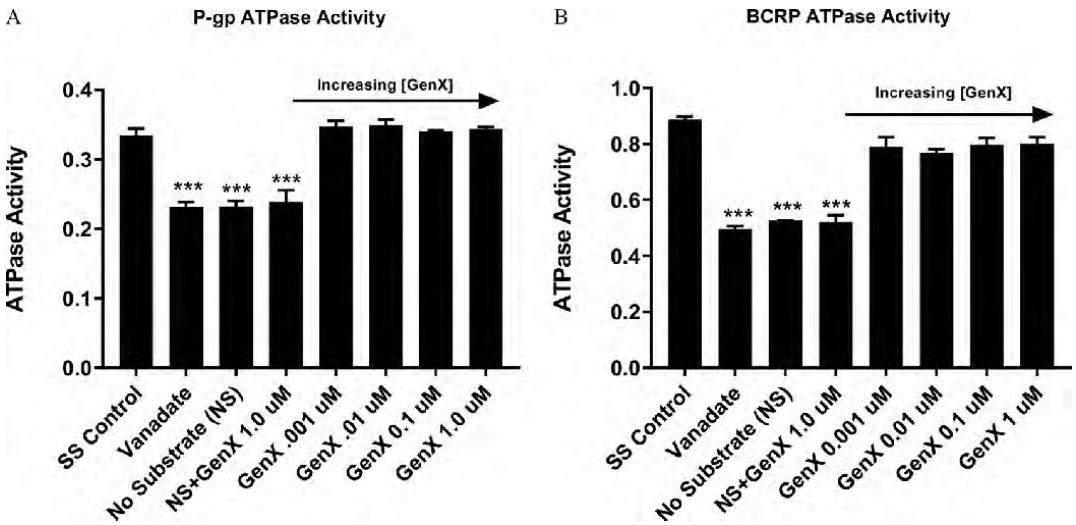

Figure 4. Ammonium 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)propanoic acid (GenX) effects on transport-associated ATPase activity *in vitro*. (A) Graph denotes rat P-glycoprotein (P-gp) ATPase activity, and (B) graph denotes rat breast cancer resistance protein (BCRP) ATPase activity. All assays were performed in triplicate. Mean ± standard error (SE) is shown. SE and significance were determined by one-way analysis of variance (ANOVA) and Tukey multiple comparison. Note: NS, no substrate added; SS control, substrate-stimulated vehicle control; vanadate (1.25 mM), ATPase inhibitor. Significance is as compared to control unless otherwise specified: $^{***}p < 0.001$.

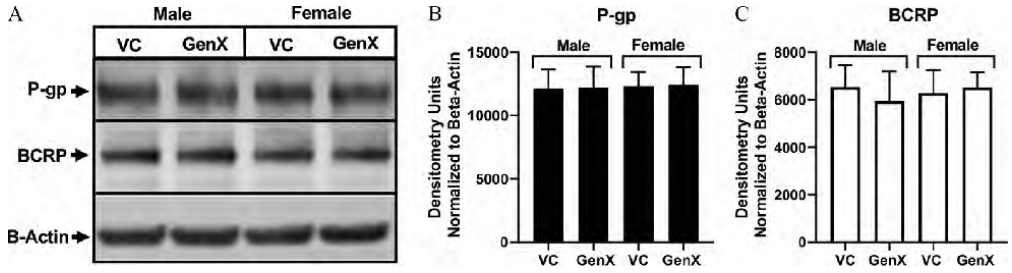

Figure 5. P-glycoprotein (P-gp) and breast cancer resistance protein (BCRP) protein levels after ammonium 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)propanoic acid (GenX) treatment. Representative Western blotting (left, Panel A) determined P-gp and BCRP protein levels in GenX- and vehicle control–treated Hsd:Sprague-Dawley® (SD®) rats ($n = 6$) brain capillary membrane lysates. Mean values are from three independent experiments. P-gp, Panel B and BCRP, Panel C levels in Western blotting (right) were determined by protein densitometry (ImageJ software) normalized to actin levels to remove sample loading variabilities. Mean ± standard error (SE) is shown. SE and significance were determined by one-way analysis of variance (ANOVA) and Tukey multiple comparison. Significance is as compared to controls.

BCRP transport activity in both male (Figure 7A,C) and female (Figure 7B,D) rat brain capillaries.

Cytotoxicity of GenX in Human Cells

To determine if the GenX effects we observed in rats occurred in human cells, we performed cell growth inhibition assays on two human-derived cell lines (NCI/ADR-RES and MX-MCF-7). NCI/ADR-RES are human-derived ovarian cells that express higher levels of P-gp relative to BCRP. MX-MCF7 are human-derived mammary epithelial cells that express higher levels of BCRP relative to P-gp. To assess the toxicity of GenX, each line was grown in media with increasing concentrations of GenX (10^{-8}–10^{-5} M), and percent cell survival was determined. We saw no change in percent cell survival for either line grown in the presence of GenX (Figure S3). Having established that GenX was not toxic to either cell line, we grew each for 72 h in complete media with or without 100 nM GenX and a cytotoxic substrate. The P-gp substrate Adriamycin (10^{-8}–10^{-5} M) was used for the NCI/ADR-RES cell line, and the BCRP substrate mitoxantrone (10^{-9}–10^{-4} M) was used with the MX-MCF-7 cell line. We reasoned if GenX inhibited P-gp or BCRP transport, it would increase substrate toxicity and significantly reduce cell survival. Shown in Figure 8A, cotreatments with 100 nM GenX and 10 μM Adriamycin reduced NCI/ADR-RES cell survival from 85% (no GenX) to 45% (with GenX). Similarly, cotreating MX-

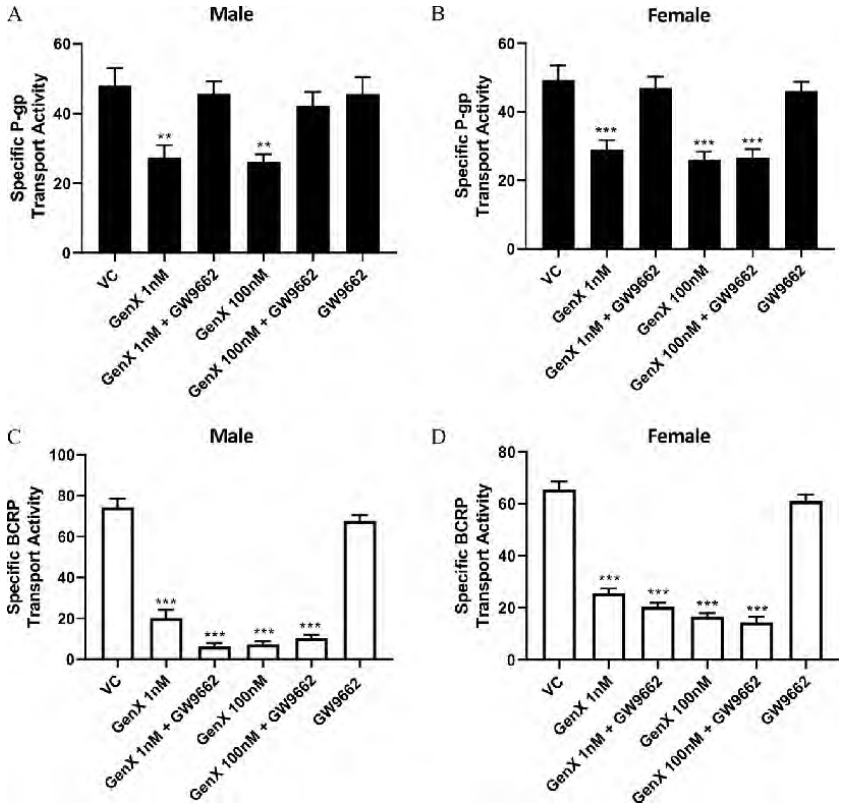

Figure 6. Inhibition of peroxisome proliferator–activated receptor gamma (PPARγ) in the ammonium 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)propanoic acid (GenX)–treated brain capillaries from six rats. P-glycoprotein (P-gp) transport activity in (A) male and (B) female rat brain capillaries treated with 1 and 100 nM GenX with or without the peroxisome PPARγ inhibitor GW9662 (50 nM). Breast cancer resistance protein (BCRP) transport activity in (C) male and (D) females rat brain capillaries treated with 1 and 100 nM GenX with or without the PPARγ inhibitor GW9662 (50 nM). Mean ± standard error (SE) is shown. SE and significance were determined by one-way analysis of variance (ANOVA) and Tukey multiple comparison. Significance was determined by comparing treatment to its respective vehicle control: $^{**}p < 0.01$; $^{***}p < 0.001$.

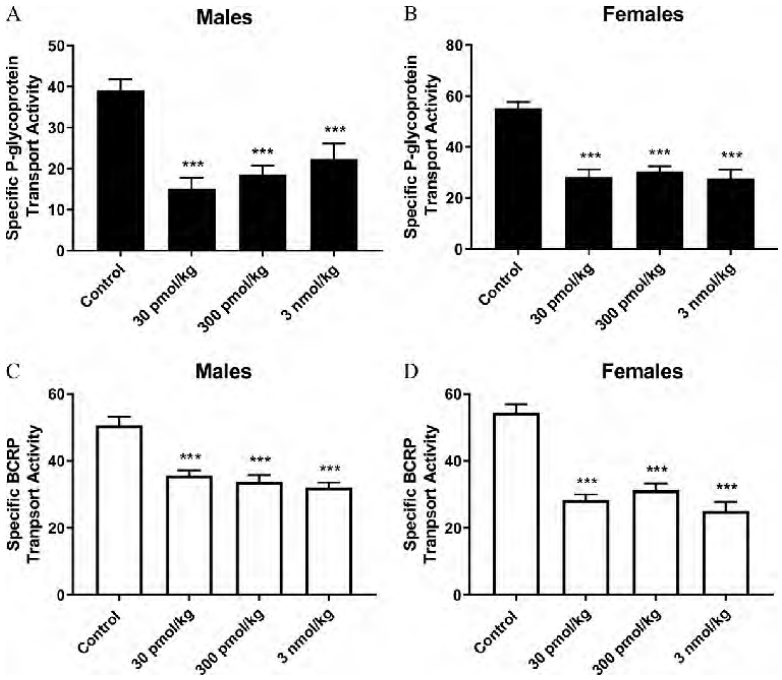

Figure 7. Dosing ammonium 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)propanoic acid (GenX) *in vivo* by oral gavage. *Ex vivo* determination of P-glycoprotein (P-gp) transport activity in (A) males and (B) females and breast cancer resistance protein (BCRP) transport activity in (C) males and (D) females following *in vivo* dosing of rats with GenX. Mean ± standard error (SE) is shown. SE and significance were determined by one-way analysis of variance (ANOVA) and Tukey multiple comparison. Significance is as compared to control: $^{***}p < 0.001$. Each dose group contained capillaries isolated from five rats.

MCF-7 human cells with 100 μM mitoxantrone and 100 nM GenX significantly reduced cell survival from 63% (no GenX) vs. 37% (with GenX).

Discussion

In the watershed of the Cape River in North Carolina, GenX is a contaminant in finished drinking water and has the capacity to alter important biological signaling pathways (Hopkins et al. 2018; Sun et al. 2016). In this report, we investigated the effects of GenX on the expression and activity of three important ABC efflux transporters (P-gp, BCRP, and MRP2) of the BBB. BBB function relies on the concerted action of endothelial cell tight junctions and luminal efflux transporters to limit the paracellular and transcellular passage of harmful xenobiotics and endogenous metabolites into the brain (Daneman 2012). We showed that exposing rat brain capillaries *ex vivo* to low nanomolar concentrations of GenX rapidly reduced the transport activity of P-gp and BCRP but not MRP2. The lack of change in MRP2 transport activity indicates that GenX affects the ABC transporters of the BBB selectively. Furthermore, it shows that the GenX treatments are not causing capillary leakage. If GenX caused capillary leakage, luminal fluorescence would decrease and measurable transport activity for MRP2 would be reduced.

To complement our *ex vivo* GenX treatments in isolated capillaries, we dosed rats with GenX by oral gavage at 30 pmol/kg,

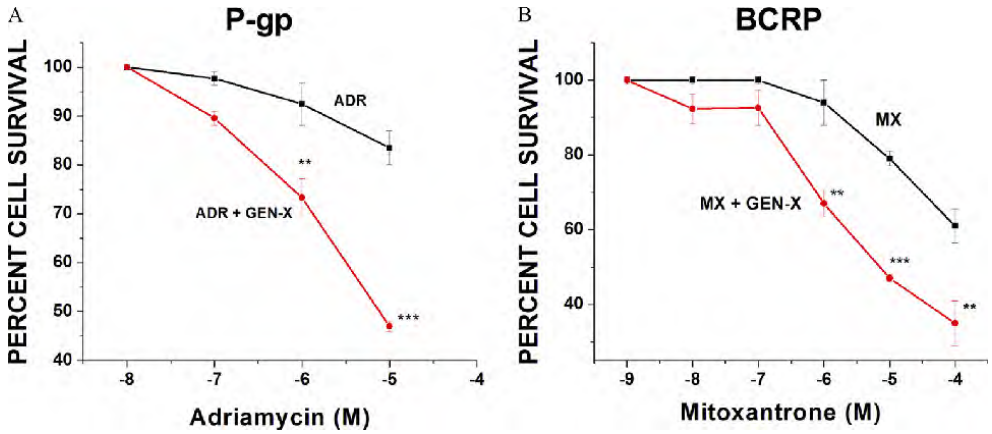

Figure 8. Ammonium 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)propanoic acid (GenX) toxicity *in vitro*. (A) Graph representing the percent of NCI/ADR-RES cells surviving 72-h Adriamycin (0.01–10 μM) exposures with (red line) or without (black line) GenX (100 nM). (B) Graph representing the percent of MX-MCF-7 cells surviving 72-h mitoxantrone (0.001–100 μM) exposures with (red line) or without (black line) GenX (100 nM). Values represent three separate experiments carried out in triplicate. Mean ± standard error (SE) is shown. SE and significance were determined by one-way analysis of variance (ANOVA) and Tukey multiple comparison. Significance is as compared to control: $^{**}p < 0.01$; $^{***}p < 0.001$.

300 pmol/kg, or 3 μmol/kg and measured their P-gp and BCRP transport activity *ex vivo*. We found that *in vivo* exposure to GenX also lowered P-gp and BCRP transport levels at all three dose groups in both male and female rats.

Our reversibility experiments using rat brain capillaries indicated that GenX inhibition of P-gp and BCRP transport is mechanistically different. P-gp transport levels returned to control levels after GenX removal in both sexes. However, BCRP transport did not return to control levels for either sex after GenX removal. We also noted significant differences between P-gp and BCRP in our PPARγ inhibitor studies. For both sexes, the PPARγ antagonist, GW9662, blocked the 1-nM GenX reductive effect on P-gp transport. Interestingly, GW9662 blocked the 100-nM GenX-dependent reductions of P-gp transport in males but not in females. This may result from differences in reported sex-specific expression levels of PPARγ (Bagal and Bungay 2014). More work is needed to fully understand this difference. Lastly, we observed no effect of GW9662 on GenX-dependent reductions of BCRP transport. Our findings suggest that nanomolar levels of GenX inhibit P-gp and BCRP transport by different mechanisms and implicates the requirement for PPARγ in the reductive effect of GenX on P-gp transport.

Data from the *in vitro* purified ATPase assay show that GenX at concentrations between 1 and 1,000 nM did not alter the levels of ATPase activity associated with P-gp or BCRP transport. They also suggest that GenX did not inhibit transport through direct contact with the P-gp or BCRP transporter proteins; therefore, we conclude that GenX is not a substrate inhibitor or an ATPase inhibitor for either transporter.

Our Western blotting data indicate that protein turnover/degradation was not a contributing factor to the GenX-dependent decreases in P-gp or BCRP transport. Quantitation from three independent protein blots showed that 4-h treatment of 100 nM GenX did not significantly alter P-gp or BCRP protein levels. The absence of changes in P-gp or BCRP protein levels by GenX treatments lends support to the idea that GenX affects transport activity and not protein expression. These experiments cannot eliminate the possibility that protein subcellular localization is contributing to GenX inhibition of P-gp or BCRP transport.

Although this work is largely focused on the transporters of the BBB, P-gp and BCRP are expressed in many human tissues, where they contribute to barrier functions of the body, e.g., testis, retina, and placenta (Liu 2019). Independent of the biological barriers, P-gp and BCRP function in the liver and kidney to remove drugs, harmful toxicants, and metabolites from the body (Leslie et al. 2005). Clinically, they are important in the absorption and distribution of prescription drugs. In addition, their activity contributes to chemoresistance in cancer therapies (Gottesman and Ling 2006; Qosa et al. 2015). They also serve to protect the brain and limit neurotoxicity during prolonged cancer chemotherapies, thus reducing the risk of therapy-related cognitive dysfunctions (Leslie et al. 2005; Miller 2015). To extend the relevance to humans and increase the importance of our findings in rats, we showed that GenX alone was not toxic to two human cell lines; however, when co-dosed with cytotoxic substrates for P-gp or BCRP, respectively, substrate toxicity increased. These data suggest that GenX can inhibit P-gp and BCRP transport in human cells.

Presently, there are no blood or urine concentration data available in the U.S. population, even though humans were likely exposed to GenX in finished drinking water. In 2017, finished drinking water from eastern North Carolina wastewater treatment plants (Pender County and Sweeney, North Carolina) contained concentrations of GenX at 340–1,100 ppt ($\sim$1–3 nM). Additional human exposures to GenX occur through contaminated air and food. The current provisional health goal for GenX in North Carolina is 140 ng/L or $\sim$0.4 nM (Hopkins et al. 2018), which is four times higher than our lowest effect level in male rats. Based on an acute and chronic aquatic toxicity study, GenX was not toxic and did not bioaccumulate in any of the species tested (*Daphnia*, trout, carp, and algae) (Hoke et al. 2016). However, GenX is persistent in the environment, and this study and others show it interacts with signaling molecules to produce important biological changes (Conley et al. 2019; Li et al. 2019).

In summary, our findings in rats suggest that low nanomolar levels of GenX can affect BBB function by inhibiting two important transporters: P-gp and BCRP. It also implicates the involvement of PPARγ for the effect of GenX on P-gp. This work is significant because inhibition of P-gp and BCRP transport function at the BBB can increase the risk of brain exposure to toxic xenobiotic agents (Roulet et al. 2003). Additionally, inhibition of P-gp and BCRP in the intestine, liver, and kidney can lead to significant changes in the absorption and elimination of harmful xenobiotics, drugs, and metabolites.

Acknowledgments

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EXHIBIT C-70

From:	James R Hoover
To:	Robert W Rickard
CC:	
BCC:	
Sent Date:	2010-06-29 16:55:21:000
Received Date:	2010-06-29 16:55:21:000
Subject:	Biomonitoring Results to EPA
Attachments:	Final PMN-08-509 Att128 Serum Results 07.31.08.doc

Bobby....here it is, rgds, Jim

Final PMN-08-509 Att128 Serum Results 07.31.08.doc

Attachment 128

Occupational Blood Serum Sampling

During a 6-day plant scale R&D test involving PMN substance P-08-509, occupational blood serum samples were collected for the purpose of assessing the effectiveness of industrial hygiene controls and further evaluating potential biopersistence. This plant scale R&D test was conducted at the DuPont Washington Works facility in West Virginia.

More specifically, blood serum samples were collected from 12 employees at the DuPont Washington Works facility who volunteered for testing. Of the 12 employees who were tested, one employee (Worker ID 1) was involved with research on the PMN substance and other potential alternatives for PFOA.[1] The remaining 11 employees worked various shifts for the plant scale R&D test. Of these 11 employees, 1 employee (Worker ID 2) was not an operator during the test, while the remaining 10 employees were operators during the test. Personal protective equipment (PPE) worn by the operators during the test is consistent with that described on PMN P-08-509 page numbers 109, 110, and 117-119.

Serum samples were analyzed for 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)propionoate anion and results are reported below, in Table 1, as the ammonium salt form [P-08-509]. Please note that this analysis does not differentiate whether the anion originates from the acid form [P-08-508] or the salt form [P-08-509]. The analytical method used was acetonitrile protein precipitation followed by LC/MS/MS.[2] Analysis was conducted by DuPont Haskell Global Centers for Health and Environmental Sciences, Newark, Delaware.

Serum samples were to be collected in 3 draws, as described below.

1st Draw: 1st Draw samples were taken prior to the start of the plant scale R&D test to establish a baseline.

2nd Draw: 2nd Draw samples were to be taken during the last shift worked by the employee during the plant scale R&D test.

3rd Draw: 3rd Draw samples were to be taken 54 to 66 hours after the 2nd Draw sample was taken, where possible.

The actual time between draws is provided in Table 1 below.

A 4th Draw sample was taken for the one employee having a 3rd Draw sample result above the quantification limit. This sample, taken 550 hours after the 3rd Draw sample, was non-detect.

[1] This employee was not involved in the plant scale R&D test. Testing was conducted per request of the employee.
[2] See Document 1, attached, for details on the analytical method.

Attachment 128 (continued)

Table 1

Occupational Blood Serum Sample Results
Reported as PMN Substance P-08-509

Worker ID	1st Draw (ng/ml)	2nd Draw (ng/ml)	Time between 1st & 2nd Draw (hrs)	3rd Draw (ng/ml)	Time between 2nd & 3rd Draw (hrs)	4th Draw (ng/ml)	Time between 3rd & 4th Draw (hrs)
1	<1	No test	-	No test	-	No test	-
2	ND	<1	93.5	<1	65	No test	-
3	ND	ND	48.5	No test	-	No test	-
4	ND	ND	83	No test	-	No test	-
5	ND	<1	95.5	ND	68	No test	-
6	ND	ND	83	No test	-	No test	-
7	ND	2	82.5	2	63	ND	550
8	ND	ND	94	No test	-	No test	-
9	ND	<1	12	ND	26	No test	-
10	ND	ND	12	No test	-	No test	-
11	ND	<1	11	ND	60	No test	-
12	<1	<1	60	<1	56	No test	-

ND = not detected, with a detection limit of approximately 0.3 ng/ml
<1 = detected but not quantifiable, with a quantification limit of 1 ng/ml
No test = a sample was not received for analysis
Analysis was conducted by DuPont Haskell Global Centers for Health and Environmental Sciences, Newark, DE
Analytical method was acetonitrile protein precipitation followed by LC/MS/MS
Hours between draws are rounded to the nearest half-hour

Attachment 128 (continued)

Document 1

Analytical Method – LC/MS/MS

2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)propionoate anion
HPLC Instrument: Agilent Model 1200
MS Instrument: Applied Biosystems API 4000
LC Parameters:
Column: Zorbax RX-C8; 150 x 2.1 mm with 5 micron particle size
Mobile Phase: A: 0.15% acetic acid and 0.15% triethyl amine in HPLC grade water
B: 0.15% acetic acid and 0.15% triethyl amine in acetonitrile
Column Temperature: 35 °C
Injection Volume: 10.0 µL
MS Parameters:
Ion Source: Turbo Spray, Negative Ion
Temperature (TEM): 450
Dwell 300 msec
Curtain Gas Flow (CUR): 50.0
GS1: 11
GS2: 70
IonSpray (IS) Voltage: -4500
CAD 10.0
EP -10.0
Quadrupole Resolution: Quad. 1: Unit
Quad. 3: Unit

MRM Settings	**Q1 Mass**	**Q3 Mass**	**DP**	**CE**	**CXP**
	329.00	285.00	-20	-10	-5

HPLC Gradient	**Step Total Time (min)**	**Flow Rate (uL/min)**	**A(%)**	**B(%)**
0	0.00	250	60.0	40.0
1	5.00	250	60.0	40.0
2	5.01	350	10.0	90.0
3	9.00	350	10.0	90.0
4	9.01	350	60.0	40.0
5	19.00	350	60.0	40.0
5	19.01	250	60.0	40.0
5	20.00	250	60.0	40.0

EXHIBIT C-71

Environmental Science & Technology

ARTICLE

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Trends in Exposure to Polyfluoroalkyl Chemicals in the U.S. Population: 1999–2008†

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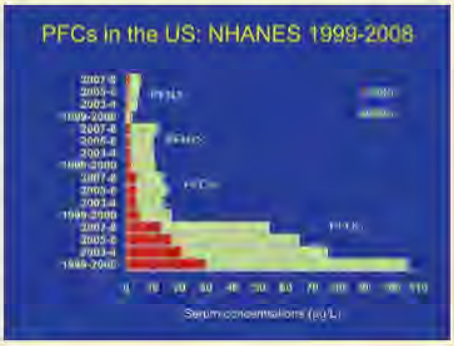
Supporting Information

ABSTRACT: Since 2002, practices in manufacturing polyfluoroalkyl chemicals (PFCs) in the United States have changed. Previous results from the National Health and Nutrition Examination Survey (NHANES) documented a significant decrease in serum concentrations of some PFCs during 1999–2004. To further assess concentration trends of perfluorooctane sulfonate (PFOS), perfluorooctanoate (PFOA), perfluorohexane sulfonate (PFHxS), and perfluorononanoate (PFNA), we analyzed 7876 serum samples collected from a representative sample of the general U.S. population ≥ 12 years of age during NHANES 1999–2008. We detected PFOS, PFOA, PFNA, and PFHxS in more than 95% of participants. Concentrations differed by sex regardless of age and we observed some differences by race/ethnicity. Since 1999–2000, PFOS concentrations showed a significant downward trend, because of discontinuing industrial production of PFOS, but PFNA concentrations showed a significant upward trend. PFOA concentrations during 1999–2000 were significantly higher than during any other time period examined, but PFOA concentrations have remained essentially unchanged during 2003–2008. PFHxS concentrations showed a downward trend from 1999 to 2006, but concentrations increased during 2007–2008. Additional research is needed to identify the environmental sources contributing to human exposure to PFCs. Nonetheless, these NHANES data suggest that sociodemographic factors may influence exposure and also provide unique information on temporal trends of exposure.



INTRODUCTION

Polyfluoroalkyl chemicals (PFCs) have been manufactured for over 60 years and used in many industrial and commercial applications.[1,2] Some PFCs, including perfluorooctane sulfonate (PFOS) and perfluorooctanoate (PFOA), are persistent compounds.

Because of the widespread exposure to PFCs in wildlife and people and the potential adverse health impacts associated with such exposures,[1,3−5] in 2002, 3M, the main manufacturer of PFOS worldwide, discontinued the production of PFOS precursors and related compounds in the United States; however, other manufacturers still produce PFOS abroad.[6] Furthermore, PFOA, its salts, and precursors are still manufactured, although efforts exist to limit emissions of PFOA into the environment.[2,7]

These efforts appear to have reduced exposure to some of these PFCs in the environment[8−10] and in people.[11−13] In the United States, general population data from the National Health and Nutrition Examination Survey (NHANES), conducted by the National Center for Health Statistics (NCHS), Centers for Disease Control and Prevention (CDC)[11,14] are reflective of this reduced exposure. Specifically, we reported before that in the six-year period covered by NHANES 1999–2000 and 2003–2004, serum concentrations of PFOS and PFOA showed a downward trend.[11] The present manuscript builds on those findings with two main objectives: (1) to assess whether the trend in exposure to PFOS, PFOA, and two additional PFCs, perfluorononanoate (PFNA) and perfluorohexane sulfonate (PFHxS), has continued since 2004, and (2) to replicate, update, and expand upon our earlier work[11,14] by examining exposure to select PFCs by use of the most recent NHANES data from 2005–2008.

MATERIALS AND METHODS

Sera analyzed for 12 PFCs was obtained from 7876 participants aged ≥12 years from NHANES 1999–2000 and 2003–2008. NCHS's Institutional Review Board approved the NHANES protocol. All participants (parents/guardians for participants <18 years of age) provided informed written consent. Details regarding the analytical procedures used have been published[11] and are publicly available at http://www.cdc.gov/nchs/nhanes.htm and in Supporting Information (SI) Table S1.

We performed the statistical analyses using SAS (version 9.2, SAS Institute, Cary, NC) and SUDAAN (version 10, Research

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Table 1. Geometric Mean and Selected Percentiles of Perfluorooctanesulfonate (PFOS) Concentrations in Serum (in ng/mL) for the U.S. Population 12 Years of Age and Older: Data from NHANES 1999–2008 [11,14a]

	survey years	geometric mean (95% confidence limit)		selected percentile (95% confidence interval) 50th		75th		90th		95th		sample size	weighted detection percent
total	99–00	**30.4**	(27.1–33.9)	**30.2**	(27.8–33.8)	**43.5**	(37.5–47.3)	**57.0**	(50.2–71.7)	**75.6**	(58.1–97.5)	1562	100
	03–04	**20.7**	(19.2–22.3)	**21.2**	(19.8–22.4)	**30.0**	(27.5–33.0)	**41.3**	(35.6–50.0)	**54.6**	(44.0–66.5)	2094	99.9
	05–06	**17.1**	(16.0–18.2)	**17.5**	(16.8–18.6)	**27.2**	(24.9–29.6)	**39.4**	(34.9–43.1)	**47.5**	(42.7–56.8)	2120	99.9
	07–08	**13.2**	(12.2–14.2)	**13.6**	(12.8–14.7)	**21.0**	(18.9–23.3)	**32.6**	(29.4–36.2)	**40.6**	(35.4–47.7)	2100	99.8
age group													
12–19 years	99–00	**29.1**	(26.2–32.4)	**29.4**	(26.8–34.2)	**38.9**	(35.9–45.0)	**52.7**	(45.6–56.2)	**57.4**	(52.7–66.5)	543	100
	03–04	**19.3**	(17.5–21.4)	**19.9**	(17.8–22.0)	**27.1**	(23.7–30.2)	**36.5**	(28.6–45.6)	**42.6**	(35.1–52.1)	640	100
	05–06	**15.0**	(14.3–15.7)	**14.9**	(13.6–16.6)	**22.7**	(19.7–24.9)	**30.6**	(27.8–34.1)	**38.5**	(33.0–44.6)	640	100
	07–08	**11.3**	(10.3–12.3)	**11.3**	(10.3–13.0)	**15.8**	(15.1–17.7)	**21.7**	(17.7–28.2)	**28.0**	(22.0–32.2)	357	100
20–39 years	99–00	**27.5**	(24.9–30.2)	**27.9**	(24.8–29.7)	**37.3**	(33.9–42.3)	**50.2**	(45.5–55.9)	**56.8**	(51.4–71.7)	364	100
	03–04	**18.7**	(17.3–20.1)	**18.7**	(17.7–20.5)	**27.4**	(24.9–29.7)	**36.9**	(33.6–41.3)	**48.7**	(38.6–60.8)	490	100
	05–06	**14.6**	(13.4–15.9)	**15.9**	(14.5–17.2)	**22.6**	(21.1–23.8)	**32.1**	(26.1–36.5)	**38.9**	(33.1–45.6)	542	100
	07–08	**11.4**	(10.1–12.8)	**11.9**	(11.0–13.2)	**18.4**	(15.9–20.4)	**26.0**	(21.4–35.1)	**35.1**	(28.4–40.8)	560	99.9
40–59 years	99–00	**33.0**	(28.0–38.8)	**33.6**	(28.0–38.7)	**46.6**	(37.9–57.0)	**75.2**	(47.1–98.4)	**94.3**	(58.0–131)	295	100
	03–04	**21.9**	(19.7–24.5)	**22.2**	(20.2–24.2)	**32.2**	(27.4–35.4)	**44.0**	(33.5–62.7)	**61.5**	(43.8–81.8)	387	99.9
	05–06	**17.3**	(15.5–19.3)	**17.6**	(15.9–20.0)	**28.9**	(24.8–32.0)	**37.3**	(32.9–49.6)	**49.6**	(36.7–74.5)	468	99.9
	07–08	**13.7**	(12.1–15.6)	**14.5**	(13.1–16.7)	**21.8**	(18.9–25.9)	**34.2**	(28.6–41.6)	**42.8**	(36.3–58.0)	568	99.7
60 years and older	99–00	**33.3**	(28.5–38.8)	**33.7**	(27.4–39.9)	**46.1**	(41.1–56.3)	**67.0**	(54.0–101)	**95.6**	(58.1–119)	360	100
	03–04	**23.2**	(20.8–25.9)	**23.9**	(21.0–27.3)	**34.7**	(30.1–39.3)	**50.3**	(40.8–68.9)	**71.6**	(49.6–90.0)	577	99.7
	05–06	**23.4**	(21.6–25.3)	**24.5**	(22.5–26.6)	**38.7**	(33.7–42.0)	**48.0**	(44.8–55.7)	**67.8**	(53.4–88.7)	470	99.9
	07–08	**17.2**	(16.0–18.6)	**18.2**	(16.3–19.6)	**28.3**	(26.0–31.2)	**41.1**	(36.0–45.3)	**51.5**	(43.2–67.1)	615	99.8
sex													
males	99–00	**33.4**	(29.6–37.6)	**34.8**	(31.1–37.9)	**46.1**	(41.0–50.2)	**58.3**	(50.2–78.3)	**78.3**	(58.0–108)	743	100
	03–04	**23.2**	(21.1–25.6)	**23.9**	(22.4–25.5)	**32.2**	(28.8–35.9)	**45.3**	(35.5–62.7)	**62.7**	(43.8–81.8)	1053	99.8
	05–06	**20.5**	(19.4–21.8)	**21.3**	(20.0–22.5)	**31.4**	(27.9–33.7)	**43.3**	(38.7–49.7)	**54.3**	(43.5–80.7)	1048	100
	07–08	**16.3**	(15.0–17.7)	**17.0**	(15.7–17.8)	**23.9**	(21.6–27.0)	**36.4**	(33.3–41.6)	**45.3**	(40.4–53.1)	1059	99.9
females	99–00	**28.0**	(24.6-31.8)	**27.7**	(24.5–30.2)	**38.8**	(32.7–46.0)	**55.4**	(46.3–70.2)	**75.7**	(56.1–98.4)	819	100
	03–04	**18.4**	(17.0–20.0)	**18.2**	(16.9–19.8)	**27.4**	(23.8–30.2)	**39.8**	(34.4–42.6)	**46.6**	(42.3–61.5)	1041	100
	05–06	**14.4**	(13.3–15.4)	**14.6**	(13.5–15.9)	**23.3**	(21.1–25.3)	**34.2**	(30.6–37.7)	**42.8**	(38.0–46.5)	1072	99.9
	07–08	**10.7**	(9.72–11.8)	**10.8**	(9.80–11.8)	**17.2**	(15.4–19.1)	**28.7**	(21.7–32.5)	**33.6**	(29.9–42.0)	1041	99.7
race/ethnicity													
non-Hispanic whites	99–00	**32.0**	(29.1–35.2)	**32.4**	(29.3–35.5)	**44.8**	(39.7–47.6)	**56.2**	(50.4–67.8)	**75.7**	(58.0–98.4)	529	100
	03–04	**21.4**	(19.9–23.1)	**22.0**	(20.5–23.0)	**30.2**	(27.7–33.3)	**41.7**	(35.7–49.6)	**56.3**	(44.0–70.0)	962	99.9
	05–06	**18.1**	(17.1–19.1)	**18.6**	(17.3–19.8)	**28.3**	(26.1–30.8)	**39.7**	(35.6–43.3)	**46.6**	(42.7–54.3)	935	99.9
	07–08	**13.7**	(12.7–14.8)	**14.3**	(13.2–15.5)	**21.1**	(18.7–23.7)	**32.7**	(29.7–36.0)	**40.4**	(36.1–44.9)	931	99.8
Mexican Americans	99–00	**22.7**	(19.8–25.9)	**23.7**	(20.8–27.2)	**32.9**	(27.8–39.6)	**41.7**	(36.5–53.6)	**53.6**	(41.3–72.0)	584	100
	03–04	**14.7**	(13.0–16.6)	**15.9**	(13.4–17.9)	**21.2**	(18.7–23.5)	**28.1**	(24.1–35.0)	**35.5**	(28.9–38.5)	485	100
	05–06	**11.2**	(10.3–12.2)	**11.6**	(9.90–13.3)	**17.2**	(15.4–19.3)	**24.9**	(22.0–30.3)	**31.9**	(26.4–40.6)	499	99.9
	07–08	**10.6**	(9.48–11.9)	**10.8**	(9.70–12.1)	**17.1**	(14.9–19.2)	**27.0**	(20.7–31.5)	**31.5**	(25.7–36.1)	391	100
non-Hispanic blacks	99–00	**33.0**	(26.2–41.6)	**32.0**	(24.3–45.7)	**50.6**	(37.4–62.2)	**68.8**	(62.0–75.9)	**82.8**	(68.7–114)	309	100
	03–04	**21.6**	(19.1–24.4)	**22.1**	(19.6–24.9)	**32.3**	(28.1–36.2)	**43.8**	(37.2–57.3)	**57.7**	(43.8–78.4)	538	99.7
	05–06	**18.4**	(15.6–21.8)	**19.0**	(16.7–22.3)	**27.6**	(24.3–35.5)	**45.9**	(34.4–58.1)	**57.9**	(45.0–84.4)	544	99.8
	07–08	**15.0**	(12.6–17.8)	**15.2**	(12.9–17.7)	**25.8**	(21.0–33.3)	**42.7**	(31.5–57.3)	**57.3**	(43.4–79.2)	419	99.7

[a] The 95% confidence intervals are shown in parentheses. The limits of detection were 0.2 ng/mL for survey years 1999–2000[14] and 2005–2008, and 0.4 ng/mL for 2003–2004.[11]

Table 2. Geometric Mean and Selected Percentiles of Perfluorooctanoate (PFOA) Concentrations in Serum (in ng/mL) for the U.S. Population 12 Years of Age and Older: Data from NHANES 1999–2008 [11,14a]

	survey years	geometric mean (95% confidence limit)		selected percentile (95% confidence interval) 50th		75th		90th		95th		sample size	weighted detection percent
total	99–00	**5.21**	(4.72–5.74)	**5.10**	(4.70–5.70)	**6.80**	(6.30–7.70)	**9.40**	(8.20–11.0)	**11.9**	(10.9–13.5)	1562	100
	03–04	**3.95**	(3.65–4.27)	**4.10**	(3.80–4.40)	**5.80**	(5.30–6.40)	**7.80**	(6.70–9.60)	**9.80**	(7.40–14.1)	2094	99.7
	05–06	**3.92**	(3.48–4.42)	**4.20**	(3.80–4.50)	**6.20**	(5.40–7.20)	**9.00**	(7.40–11.2)	**11.3**	(8.80–14.5)	2120	99.8
	07–08	**4.13**	(4.01–4.25)	**4.30**	(4.10–4.50)	**5.90**	(5.70–6.20)	**7.90**	(7.50–8.30)	**9.70**	(9.00–10.1)	2100	99.9
age group													
12–19 years	99–00	**5.46**	(4.98–5.99)	**5.60**	(4.80–6.10)	**6.90**	(6.20–7.50)	**9.40**	(7.70–11.0)	**11.2**	(10.2–12.5)	543	99.9
	03–04	**3.89**	(3.47–4.35)	**4.00**	(3.50–4.50)	**5.40**	(4.60–6.10)	**7.00**	(5.60–9.20)	**8.60**	(5.90–12.6)	640	99.9
	05–06	**3.59**	(3.26–3.96)	**3.80**	(3.30–4.20)	**5.40**	(4.60–5.90)	**6.90**	(6.30–7.90)	**8.40**	(7.30–10.1)	640	99.9
	07–08	**3.91**	(3.70–4.12)	**4.00**	(3.60–4.30)	**5.00**	(4.70–5.50)	**6.10**	(5.70–6.70)	**7.30**	(6.20–8.00)	357	100
20–39 years	99–00	**5.18**	(4.67–5.74)	**5.20**	(4.70–6.20)	**7.10**	(6.40–7.80)	**9.30**	(7.80–11.0)	**10.9**	(8.40–14.0)	364	100
	03–04	**3.87**	(3.57–4.19)	**4.10**	(3.70–4.50)	**5.80**	(5.40–6.20)	**7.70**	(7.30–8.40)	**9.60**	(8.40–11.1)	490	99.5
	05–06	**3.72**	(3.26–4.24)	**4.00**	(3.60–4.40)	**6.20**	(5.00–7.30)	**8.80**	(7.40–11.0)	**11.2**	(9.00–13.1)	542	99.8
	07–08	**3.99**	(3.75–4.24)	**4.20**	(3.90–4.50)	**6.10**	(5.70–6.70)	**8.00**	(7.40–8.70)	**9.60**	(8.70–10.1)	560	100
40–59 years	99–00	**5.36**	(4.66–6.16)	**5.20**	(4.50–5.90)	**6.90**	(6.00–8.70)	**9.40**	(7.70–14.1)	**13.0**	(10.0–17.7)	295	100
	03–04	**4.24**	(3.76–4.78)	**4.30**	(3.90–4.80)	**6.30**	(5.40–7.20)	**8.20**	(6.80–10.7)	**10.6**	(7.40–16.9)	387	100
	05–06	**3.85**	(3.37–4.40)	**4.20**	(3.80–4.70)	**6.10**	(5.40–7.20)	**8.60**	(7.10–10.5)	**10.3**	(8.00–13.8)	468	99.7
	07–08	**4.19**	(3.91–4.49)	**4.50**	(4.10–4.90)	**6.10**	(5.70–6.60)	**8.10**	(7.00–10.0)	**10.1**	(8.40–11.9)	568	99.7
60 years and older	99–00	**4.82**	(4.25–5.47)	**4.80**	(4.30–5.10)	**6.40**	(5.70–7.50)	**8.80**	(7.30–12.0)	**11.5**	(8.70–17.1)	360	100
	03–04	**3.65**	(3.29–4.06)	**3.90**	(3.50–4.30)	**5.50**	(4.90–6.00)	**7.30**	(6.00–9.90)	**9.50**	(6.90–14.1)	577	99.2
	05–06	**4.65**	(3.99–5.41)	**4.60**	(4.10–5.20)	**7.40**	(5.30–9.60)	**11.4**	(8.10–14.8)	**14.2**	(9.70–23.0)	470	99.8
	07–08	**4.40**	(4.16–4.66)	**4.40**	(4.10–4.70)	**6.30**	(5.80–6.70)	**8.20**	(7.50–8.80)	**9.80**	(8.20–12.7)	615	99.9
sex													
males	99–00	**5.71**	(5.17–6.31)	**6.00**	(5.40–6.40)	**7.60**	(6.80–8.40)	**10.5**	(9.00–11.8)	**12.1**	(11.0–13.1)	743	100
	03–04	**4.47**	(4.07–4.91)	**4.60**	(4.30–5.00)	**6.30**	(5.70–7.20)	**8.40**	(6.80–12.5)	**10.7**	(7.40–17.5)	1053	99.6
	05–06	**4.69**	(4.23–5.20)	**4.90**	(4.40–5.40)	**7.20**	(6.10–8.00)	**9.90**	(8.00–12.2)	**12.2**	(9.60–15.2)	1048	99.9
	07–08	**4.80**	(4.56–5.06)	**4.90**	(4.60–5.20)	**6.70**	(6.30–7.10)	**8.70**	(8.10–9.30)	**10.1**	(9.70–11.1)	1059	100
females	99–00	**4.80**	(4.32–5.34)	**4.60**	(4.20–5.00)	**6.20**	(5.60–7.00)	**8.30**	(7.50–9.90)	**11.3**	(9.20–14.4)	819	100
	03–04	**3.50**	(3.21–3.82)	**3.60**	(3.30–3.90)	**5.20**	(4.70–5.80)	**7.10**	(6.30–8.20)	**8.60**	(7.40–10.6)	1041	99.8
	05–06	**3.31**	(2.89–3.79)	**3.50**	(3.10–4.00)	**5.20**	(4.40–6.00)	**7.90**	(6.10–9.70)	**10.1**	(7.50–14.2)	1072	99.7
	07–08	**3.56**	(3.38–3.74)	**3.70**	(3.50–3.90)	**5.20**	(4.80–5.60)	**7.00**	(6.50–7.60)	**8.30**	(7.30–9.90)	1041	99.7
race/ethnicity													
non-Hispanic whites	99–00	**5.60**	(5.05–6.22)	**5.50**	(4.90–6.20)	**7.30**	(6.50–8.20)	**10.0**	(8.30–12.0)	**12.9**	(11.0–14.9)	529	100
	03–04	**4.18**	(3.85–4.53)	**4.30**	(3.90–4.70)	**6.00**	(5.50–6.70)	**7.90**	(7.20–9.20)	**9.90**	(7.60–13.3)	962	99.8
	05–06	**4.27**	(3.80–4.81)	**4.40**	(4.00–5.00)	**6.60**	(5.60–7.80)	**9.60**	(7.40–12.2)	**11.6**	(8.80–14.8)	935	99.9
	07–08	**4.38**	(4.20–4.56)	**4.60**	(4.30–4.70)	**6.10**	(5.80–6.60)	**8.20**	(7.80–8.90)	**9.90**	(9.10–10.6)	931	99.8
Mexican Americans	99–00	**3.89**	(3.58–4.21)	**4.20**	(3.70–4.60)	**5.80**	(5.20–6.20)	**7.60**	(6.40–8.00)	**8.00**	(7.70–8.90)	584	99.9
	03–04	**3.11**	(2.84–3.40)	**3.30**	(3.10–3.70)	**4.50**	(4.20–5.20)	**6.70**	(5.70–7.30)	**7.60**	(6.70–10.5)	485	100
	05–06	**2.62**	(2.33–2.95)	**2.80**	(2.50–3.30)	**4.30**	(3.80–4.70)	**5.80**	(5.30–6.70)	**7.40**	(5.90–8.10)	499	99.1
	07–08	**3.53**	(3.34–3.74)	**3.80**	(3.50–4.00)	**5.20**	(4.90–5.60)	**6.60**	(6.20–7.10)	**7.60**	(6.80–9.00)	391	100
non-Hispanic blacks	99–00	**4.80**	(4.12–5.58)	**4.80**	(4.00–5.90)	**6.50**	(5.90–7.50)	**8.80**	(7.40–11.5)	**11.1**	(9.20–14.0)	309	100
	03–04	**3.37**	(2.99–3.79)	**3.70**	(3.20–4.20)	**5.20**	(4.40–6.30)	**7.70**	(5.30–11.6)	**9.60**	(6.50–13.9)	538	99.3
	05–06	**3.27**	(2.61–4.08)	**3.70**	(3.00–4.20)	**5.50**	(4.40–6.80)	**8.10**	(6.00–11.3)	**10.4**	(7.80–12.3)	544	99
	07–08	**3.86**	(3.57–4.16)	**4.00**	(3.50–4.30)	**5.90**	(5.20–6.50)	**7.80**	(7.10–8.70)	**9.20**	(8.50–10.1)	419	99.8

[a] The 95% confidence intervals are shown in parentheses. The limit of detection was 0.1 ng/mL for all survey years: 1999–2000,[14] 2003–2004,[11] and 2005–2008.

Table 3. Geometric Mean and Selected Percentiles of Perfluorononanoate (PFNA) Concentrations in Serum (in ng/mL) for the U.S. Population 12 Years of Age and Older: Data from NHANES 1999–2008 [11,14a]

		geometric		selected percentile								sample	weighted
	survey	mean		(95% confidence interval)								size	detection
	years	(95% confidence limit)		50th		75th		90th		95th			percent
total	99–00	**.547**	(0.450-.665)	**.600**	(0.500-.600)	**.900**	(0.700–1.00)	**1.20**	(1.00–1.50)	**1.70**	(1.30–2.40)	1562	94.6
	03–04	**.966**	(0.816–1.14)	**1.00**	(0.900–1.10)	**1.50**	(1.20–1.80)	**2.30**	(1.60–4.30)	**3.20**	(1.80–7.70)	2094	98.8
	05–06	**1.09**	(0.909–1.29)	**1.10**	(0.900–1.20)	**1.70**	(1.30–2.10)	**2.60**	(1.80–4.20)	**3.60**	(2.50–5.70)	2120	99.3
	07–08	**1.49**	(1.37–1.61)	**1.50**	(1.40–1.60)	**2.10**	(1.90–2.30)	**3.20**	(2.80–3.50)	**4.00**	(3.60–4.60)	2100	99.5
age group													
12–19 years	99–00	**.470**	(0.403-.548)	**.400**	(0.400-.500)	**.700**	(0.500-.800)	**.900**	(0.800-.900)	**1.10**	(0.900–1.40)	543	96.1
	03–04	**.852**	(0.697–1.04)	**.800**	(0.700–1.00)	**1.20**	(1.00–1.60)	**1.90**	(1.20–3.70)	**2.80**	(1.30–6.30)	640	98.8
	05–06	**.929**	(0.782–1.10)	**.900**	(0.800–1.10)	**1.40**	(1.10–1.80)	**2.00**	(1.60–2.70)	**2.70**	(2.10–3.40)	640	99
	07–08	**1.42**	(1.27–1.59)	**1.40**	(1.20–1.60)	**1.90**	(1.60–2.40)	**2.50**	(2.00–3.30)	**3.10**	(2.60–4.00)	357	100
20–39 years	99–00	**.519**	(0.424-.635)	**.400**	(0.400-.500)	**.700**	(0.600-.900)	**1.10**	(0.800–1.50)	**1.50**	(1.20–2.30)	364	95.8
	03–04	**.965**	(0.827–1.13)	**1.00**	(0.800–1.10)	**1.50**	(1.30–1.70)	**2.20**	(1.70–2.80)	**2.80**	(1.90–6.10)	490	99
	05–06	**1.01**	(0.814–1.25)	**1.00**	(0.900–1.20)	**1.50**	(1.20–2.00)	**2.60**	(1.70–3.90)	**3.30**	(2.20–6.20)	542	98.8
	07–08	**1.49**	(1.34–1.65)	**1.50**	(1.40–1.70)	**2.10**	(2.00–2.30)	**3.10**	(2.70–3.50)	**4.00**	(3.30–5.00)	560	99.5
40–59 years	99–00	**.566**	(0.445-.721)	**.600**	(0.500-.700)	**1.00**	(0.700–1.20)	**1.30**	(1.00–2.20)	**1.90**	(1.30–2.40)	295	91.1
	03–04	**1.10**	(0.883–1.37)	**1.10**	(0.900–1.20)	**1.70**	(1.20–2.40)	**2.70**	(1.70–5.90)	**4.30**	(1.70–9.30)	387	99.5
	05–06	**1.14**	(0.935–1.38)	**1.10**	(0.900–1.30)	**1.80**	(1.30–2.50)	**2.60**	(1.80–4.40)	**3.50**	(2.40–5.70)	468	99.7
	07–08	**1.50**	(1.37–1.65)	**1.50**	(1.40–1.70)	**2.20**	(2.00–2.30)	**3.20**	(2.80–3.80)	**3.90**	(3.30–4.60)	568	99.3
60 years and older	99–00	**.648**	(0.521-.805)	**.500**	(0.500-.700)	**1.00**	(0.800–1.20)	**1.80**	(1.20–2.20)	**2.10**	(1.80–3.60)	360	96.8
	03–04	**.846**	(0.721-.993)	**.900**	(0.800–1.00)	**1.30**	(1.10–1.60)	**2.00**	(1.50–3.30)	**3.00**	(1.60–7.40)	577	97.4
	05–06	**1.24**	(1.03–1.49)	**1.20**	(1.10–1.30)	**1.80**	(1.50–2.50)	**3.00**	(1.90–5.60)	**4.60**	(2.40–10.0)	470	99.6
	07–08	**1.50**	(1.28–1.75)	**1.50**	(1.30–1.70)	**2.10**	(1.80–2.70)	**3.50**	(2.70–4.60)	**4.80**	(3.60–6.00)	615	99.6
sex													
males	99–00	**.600**	(0.503-.715)	**.600**	(0.500-.600)	**.800**	(0.700–1.00)	**1.30**	(1.00–1.40)	**1.70**	(1.30–2.30)	743	96
	03–04	**1.09**	(0.912–1.30)	**1.10**	(0.900–1.20)	**1.60**	(1.40–1.90)	**2.40**	(1.70–5.00)	**4.00**	(1.80–8.70)	1053	99.1
	05–06	**1.19**	(0.993–1.43)	**1.20**	(1.00–1.30)	**1.80**	(1.40–2.50)	**2.90**	(1.80–4.60)	**3.90**	(2.50–6.70)	1048	99.1
	07–08	**1.66**	(1.54–1.79)	**1.60**	(1.50–1.70)	**2.30**	(2.10–2.50)	**3.50**	(3.20–3.80)	**4.30**	(3.80–5.10)	1059	99.6
females	99–00	**.505**	(0.404-.631)	**.500**	(0.400-.500)	**.700**	(0.600-.900)	**1.30**	(0.900–1.90)	**1.70**	(1.20–2.40)	819	93.3
	03–04	**.861**	(0.721–1.03)	**.900**	(0.800–1.00)	**1.30**	(1.00–1.70)	**2.20**	(1.40–3.40)	**3.00**	(1.70–6.10)	1041	98.5
	05–06	**.992**	(0.833–1.18)	**.900**	(0.800–1.10)	**1.50**	(1.20–1.90)	**2.40**	(1.70–3.50)	**3.10**	(2.10–5.70)	1072	99.4
	07–08	**1.33**	(1.20–1.47)	**1.30**	(1.20–1.50)	**1.90**	(1.80–2.10)	**2.70**	(2.40–3.10)	**3.40**	(3.10–4.30)	1041	99.4
race/ethnicity													
non-Hispanic whites	99–00	**.561**	(0.461-.683)	**.600**	(0.500-.600)	**.900**	(0.700–1.10)	**1.20**	(1.00–1.70)	**1.70**	(1.30–2.30)	529	95.8
	03–04	**.963**	(0.826–1.12)	**.900**	(0.900–1.10)	**1.50**	(1.20–1.70)	**2.30**	(1.60–3.60)	**3.00**	(1.80–6.20)	962	99.2
	05–06	**1.10**	(0.914–1.31)	**1.10**	(0.900–1.20)	**1.70**	(1.30–2.10)	**2.60**	(1.80–4.20)	**3.50**	(2.40–5.70)	935	99.3
	07–08	**1.48**	(1.35–1.64)	**1.50**	(1.40–1.60)	**2.10**	(1.80–2.30)	**3.10**	(2.60–3.70)	**3.90**	(3.40–4.70)	931	99.6
Mexican Americans	99–00	**.336**	(0.288-.392)	**.400**	(0.300-.400)	**.400**	(0.400-.600)	**.600**	(0.600-.800)	**.900**	(0.700–1.20)	584	88.5
	03–04	**.689**	(0.586-.809)	**.700**	(0.600-.900)	**1.10**	(0.900–1.40)	**1.60**	(1.30–2.00)	**2.00**	(1.60–2.80)	485	96.5
	05–06	**.821**	(0.710-.951)	**.800**	(0.700-.900)	**1.20**	(1.10–1.40)	**1.60**	(1.30–2.30)	**2.30**	(1.50–3.20)	499	98.8
	07–08	**1.34**	(1.18–1.53)	**1.40**	(1.20–1.60)	**2.00**	(1.70–2.20)	**2.60**	(2.20–3.30)	**3.30**	(2.60–4.00)	391	99.9
non-Hispanic blacks	99–00	**.759**	(0.605-.953)	**.600**	(0.500-.800)	**1.20**	(0.800–1.70)	**1.80**	(1.40–2.40)	**2.30**	(1.80–2.90)	309	99.5
	03–04	**1.14**	(0.834–1.54)	**1.10**	(0.900–1.40)	**1.70**	(1.20–2.90)	**3.20**	(1.50–6.50)	**4.70**	(2.10–9.30)	538	99.1
	05–06	**1.17**	(0.916–1.50)	**1.20**	(0.900–1.40)	**1.60**	(1.30–2.50)	**2.60**	(1.70–5.60)	**4.40**	(2.40–8.20)	544	99.6
	07–08	**1.65**	(1.50–1.80)	**1.70**	(1.50–1.80)	**2.30**	(2.10–2.60)	**3.40**	(2.80–4.10)	**4.10**	(3.40–5.70)	419	99.6

[a] The 95% confidence intervals are shown in parentheses. The limit of detection was 0.1 ng/mL for all survey years: 1999–2000,[14] 2003–2004,[11] and 2005–2008.

Table 4. Geometric mean and selected percentiles of perfluorohexanesulfonate (PFHxS) concentrations in serum (in ng/mL) for the U.S. population 12 years of age and older: Data from NHANES 1999–2008 [11,14a]

	survey years	geometric mean (95% confidence limit)		selected percentile (95% confidence interval) 50th		75th		90th		95th		sample size	weighted detection percent
total	99–00	**2.13**	(1.91–2.38)	**2.10**	(1.80–2.30)	**3.30**	(3.00–3.80)	**5.70**	(5.10–6.70)	**8.70**	(7.00–10.0)	1562	99.6
	03–04	**1.93**	(1.73–2.16)	**1.90**	(1.70–2.10)	**3.30**	(2.80–3.90)	**5.90**	(4.80–7.20)	**8.30**	(7.10–9.70)	2094	98.3
	05–06	**1.67**	(1.42–1.98)	**1.80**	(1.50–2.10)	**3.20**	(2.60–4.00)	**5.40**	(4.40–7.10)	**8.30**	(5.80–11.9)	2120	96.9
	07–08	**1.96**	(1.76–2.17)	**2.00**	(1.80–2.10)	**3.50**	(3.10–4.00)	**5.90**	(4.90–8.30)	**9.80**	(6.10–15.2)	2100	99.2
age group													
12–19 years	99–00	**2.69**	(2.14–3.39)	**2.40**	(2.00–3.70)	**5.00**	(3.50–6.50)	**7.90**	(5.70–12.9)	**12.9**	(6.80–15.6)	543	99.9
	03–04	**2.44**	(2.05–2.90)	**2.40**	(1.80–3.20)	**4.90**	(4.00–6.30)	**9.50**	(6.80–12.5)	**13.3**	(9.90–19.6)	640	98.8
	05–06	**2.09**	(1.74–2.52)	**2.40**	(2.00–2.90)	**4.30**	(3.70–5.40)	**9.50**	(6.40–13.1)	**14.1**	(9.80–17.8)	640	96
	07–08	**2.39**	(2.09–2.75)	**2.30**	(1.80–2.70)	**4.30**	(3.70–5.70)	**9.90**	(6.50–12.5)	**15.9**	(11.1–21.5)	357	99.7
20–39 years	99–00	**1.98**	(1.69–2.31)	**1.80**	(1.50–2.30)	**3.30**	(2.70–3.90)	**5.70**	(4.70–6.90)	**8.40**	(5.70–10.6)	364	99.4
	03–04	**1.77**	(1.56–2.01)	**1.80**	(1.50–2.10)	**2.90**	(2.60–3.50)	**4.80**	(3.90–6.10)	**6.70**	(4.90–9.40)	490	97.8
	05–06	**1.58**	(1.31–1.90)	**1.80**	(1.40–2.20)	**3.30**	(2.90–4.00)	**5.40**	(4.40–6.50)	**7.80**	(5.20–12.5)	542	95.7
	07–08	**1.65**	(1.47–1.85)	**1.60**	(1.40–2.00)	**3.00**	(2.70–3.30)	**5.10**	(3.70–7.40)	**7.40**	(4.60–12.0)	560	99.6
40–59 years	99–00	**2.07**	(1.83–2.33)	**2.00**	(1.70–2.30)	**3.20**	(2.90–3.90)	**5.00**	(4.00–5.80)	**6.70**	(5.20–7.90)	295	99.8
	03–04	**1.85**	(1.59–2.15)	**1.70**	(1.50–2.10)	**3.20**	(2.30–4.50)	**5.50**	(4.30–6.90)	**6.90**	(5.50–8.20)	387	98.6
	05–06	**1.52**	(1.25–1.85)	**1.70**	(1.30–2.00)	**2.90**	(2.30–3.40)	**4.90**	(3.70–5.80)	**6.40**	(5.10–7.30)	468	97.6
	07–08	**1.88**	(1.57–2.25)	**1.80**	(1.60–2.10)	**3.40**	(2.60–4.30)	**5.80**	(4.20–11.2)	**11.1**	(5.10–20.5)	568	98.2
60 years and older	99–00	**2.16**	(1.89–2.48)	**2.10**	(1.90–2.40)	**3.20**	(2.60–4.00)	**5.90**	(4.40–8.80)	**9.50**	(5.20–10.6)	360	99.1
	03–04	**2.04**	(1.74–2.39)	**1.90**	(1.70–2.20)	**3.20**	(2.60–4.10)	**7.30**	(4.40–10.2)	**10.2**	(7.20–12.9)	577	98.5
	05–06	**1.88**	(1.54–2.30)	**1.90**	(1.60–2.20)	**3.10**	(2.30–4.40)	**5.60**	(3.80–9.30)	**9.30**	(4.90–13.0)	470	97.9
	07–08	**2.42**	(2.20–2.68)	**2.40**	(2.20–2.70)	**3.80**	(3.50–4.40)	**6.50**	(5.50–7.50)	**9.10**	(6.80–11.7)	615	99.8
sex													
males	99–00	**2.61**	(2.26–3.00)	**2.50**	(2.20–2.90)	**4.00**	(3.40–4.50)	**6.60**	(5.30–9.40)	**10.1**	(6.90–15.0)	743	99.2
	03–04	**2.17**	(1.87–2.51)	**2.10**	(1.80–2.40)	**3.40**	(2.80–4.50)	**6.10**	(4.60–8.10)	**8.50**	(6.50–10.5)	1053	98.6
	05–06	**2.08**	(1.76–2.45)	**2.20**	(1.80–2.70)	**3.70**	(3.20–4.60)	**6.40**	(4.80–9.30)	**9.50**	(6.70–13.7)	1048	97.2
	07–08	**2.63**	(2.36–2.94)	**2.60**	(2.40–2.80)	**4.20**	(3.80–4.90)	**7.30**	(5.50–11.0)	**11.4**	(7.10–18.1)	1059	99.4
females	99–00	**1.79**	(1.56–2.06)	**1.70**	(1.30–1.90)	**2.70**	(2.30–3.50)	**5.10**	(4.10–6.70)	**8.00**	(5.70–9.00)	819	99.9
	03–04	**1.72**	(1.56–1.91)	**1.60**	(1.40–1.80)	**2.90**	(2.50–3.50)	**5.80**	(4.60–7.10)	**8.20**	(6.70–10.0)	1041	98.1
	05–06	**1.37**	(1.15–1.63)	**1.50**	(1.20–1.70)	**2.80**	(2.20–3.30)	**4.60**	(3.90–5.80)	**6.60**	(5.60–9.30)	1072	96.5
	07–08	**1.46**	(1.30–1.64)	**1.40**	(1.30–1.50)	**2.60**	(2.20–3.10)	**4.70**	(3.80–6.10)	**7.50**	(5.00–12.5)	1041	98.9
race/ethnicity													
non-Hispanic whites	99–00	**2.28**	(2.04–2.55)	**2.10**	(1.80–2.50)	**3.60**	(3.20–4.10)	**5.90**	(5.20–6.70)	**8.30**	(7.00–9.60)	529	99.8
	03–04	**2.01**	(1.77–2.27)	**1.90**	(1.70–2.20)	**3.30**	(2.80–4.10)	**6.10**	(4.70–7.80)	**8.20**	(6.90–10.1)	962	98.9
	05–06	**1.79**	(1.48–2.17)	**1.80**	(1.50–2.20)	**3.30**	(2.70–4.30)	**5.60**	(4.60–8.20)	**9.40**	(6.10–13.1)	935	97.7
	07–08	**2.13**	(1.89–2.41)	**2.10**	(1.80–2.40)	**3.70**	(3.20–4.20)	**6.40**	(5.30–8.90)	**10.5**	(6.50–15.6)	931	99.2
Mexican Americans	99–00	**1.47**	(1.12–1.92)	**1.40**	(1.00–2.00)	**2.70**	(1.80–3.80)	**4.40**	(3.30–6.90)	**6.60**	(4.60–9.50)	584	99
	03–04	**1.42**	(1.17–1.72)	**1.50**	(1.20–1.70)	**2.30**	(1.90–2.90)	**4.30**	(3.10–5.10)	**5.50**	(4.00–8.90)	485	94.8
	05–06	**1.25**	(0.884–1.76)	**1.60**	(1.20–2.10)	**2.80**	(2.50–3.40)	**4.30**	(3.60–5.20)	**6.00**	(4.60–7.20)	499	92.1
	07–08	**1.65**	(1.47–1.87)	**1.70**	(1.40–1.90)	**3.00**	(2.70–3.40)	**5.00**	(3.70–6.10)	**6.70**	(5.30–9.10)	391	99.2
non-Hispanic blacks	99–00	**2.16**	(1.59–2.93)	**2.20**	(1.30–3.00)	**3.40**	(2.70–4.70)	**6.70**	(4.30–10.7)	**10.6**	(7.20–14.3)	309	100
	03–04	**1.92**	(1.62–2.26)	**1.90**	(1.60–2.20)	**3.50**	(2.80–4.60)	**6.00**	(5.00–7.10)	**8.30**	(6.30–12.0)	538	97.5
	05–06	**1.53**	(1.13–2.07)	**1.70**	(1.40–2.20)	**3.30**	(2.50–4.10)	**5.40**	(3.90–7.50)	**7.50**	(5.30–11.0)	544	96.5
	07–08	**1.98**	(1.55–2.52)	**2.10**	(1.80–2.50)	**3.90**	(2.80–5.40)	**7.90**	(4.20–12.5)	**11.1**	(5.90–17.2)	419	98.9

[a] The 95% confidence intervals are shown in parentheses. The limits of detection were 0.1 ng/mL for 1999–2000[14] and 2005–2008, and 0.3 ng/mL for 2003–2004[11]

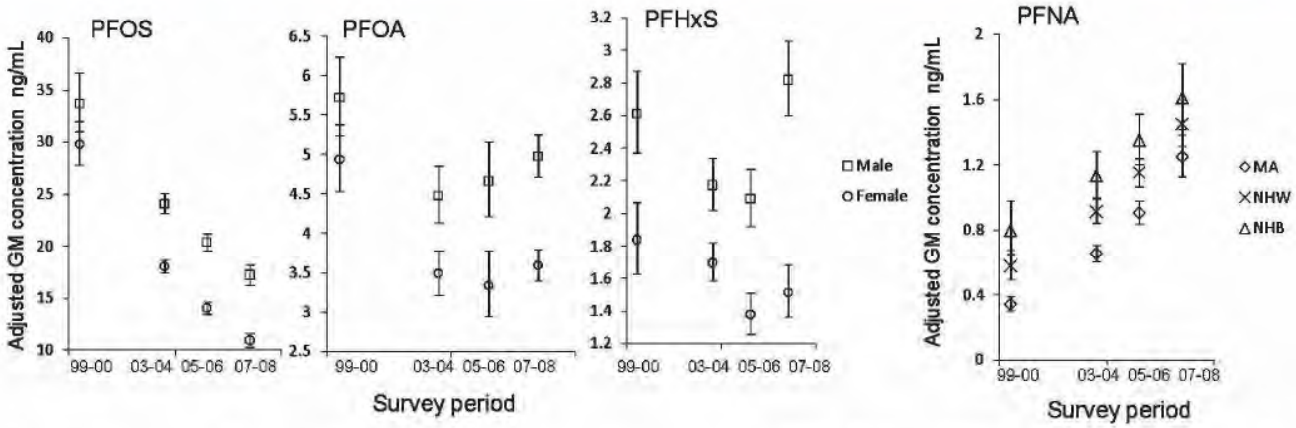

Figure 1. Temporal trend for the model adjusted geometric mean serum concentrations of PFOS, PFOA, and PFHxS by sex and of PFNA by race. The error bars represent the 95% confidence intervals. MA (Mexican American), NHW (non-Hispanic white), NHB (non-Hispanic black).

Triangle Institute, Research Triangle Park, NC). For each NHANES two-year cycle, SUDAAN calculates variance estimates after incorporating the sample population weights, designed for the one-third subset of the full survey. The weights account for unequal selection probabilities and planned oversampling of certain subgroups resulting from the complex multistage area probability design of NHANES. We categorized race/ethnicity on the basis of self-reported data as non-Hispanic black, non-Hispanic white, and Mexican American. We included persons not defined by these racial groups only in the total population estimate. We stratified age, reported in years at the last birthday, in four groups (12−19, 20−39, 40−59, and 60 years and older). We estimated the weighted percentage of detection and calculated weighted geometric means and percentiles for the PFCs serum concentrations (in ng/mL). For concentrations below the limit of detection (LOD), we used a value equal to LOD/$\sqrt{2}$. Because PFC concentrations were not normally distributed, we used the natural log transformation. Statistical significance was set at $P < 0.05$.

We used analysis of covariance to examine the influence of various demographic parameters and survey cycle on the log-transformed serum concentrations of PFOS, PFOA, PFHxS, and PFNA. For multiple regressions, we calculated the least-squares geometric means (LSGM) using SUDAAN and compared them for each categorical variable. The variables included in the initial model were sex, age, race/ethnicity, and survey period (coded 1, 3, 4, and 5 as continuous variables). For PFOA and PFHxS, we observed a curve-linear relationship between survey period and concentrations (a significant quadratic term in survey period). To calculate the LSGM, we treated survey period as a categorical variable in the regression models. We assessed all possible two-way interaction terms in each model.

For each analyte, to reach the final reduced model, we followed a backward elimination approach with a threshold of $P < 0.05$ for retaining the variable in the model, using Satterwaite adjusted F statistics. We evaluated for potential confounding by adding each of the excluded variables back into the final model one by one and examining changes in the β-coefficients of the statistically significant main effect. If adding one of these excluded variables caused a change in a β-coefficient by ≥10%, we readded the variable to the model.

RESULTS AND DISCUSSION

We report the distribution of serum concentrations of PFOS, PFOA, PFNA, and PFHxS, the four most commonly detected PFCs, stratified by age, sex, and race/ethnicity in Tables 1−4. We detected PFOS, PFOA, PFNA, and PFHxS in more than 95% of the samples during the four NHANES periods examined. These data confirmed that serum concentrations of these PFCs are still measurable in the U.S. general population in 2007−2008. Furthermore, the ranges of concentrations of PFOS, PFOA, and PFHxS during NHANES 2005−2006 and 2007−2008 agree with biomonitoring data from select populations from the United States in 2006,[12] from several European countries in 2005−2006[3] and 2005−2009,[13,15,16] and from China in 2009.[17]

We detected eight other PFCs—perfluorooctane sulfonamide, 2-(*N*-ethyl-perfluorooctane sulfonamido) acetate, 2-(*N*-methyl-perfluorooctane sulfonamido) acetate, perfluorobutane sulfonate, perfluoroheptanoate, perfluorodecanoate, perfluoroundecanoate, and perfluorododecanoate—less frequently. Their geometric means and selected percentile concentrations are given in SI Tables S2−S8, along with a discussion about their detection frequencies. For these PFCs, we did not perform additional statistical analyses.

We show the regression model results for PFOS, PFOA, PFNA and PFHxS in SI Tables S9−S12. LSGM concentrations provide geometric mean estimates after adjustment for the model covariates (SI Table S13). We show the statistical significance values of the final models in the comparison of these LSGM concentrations in SI Table S14.

Regardless of survey period and of age, males had significantly higher ($P < 0.01$) PFOS LSGM concentrations than did females. Interestingly, PFOS LSGM concentrations have decreased since NHANES 1999−2000 (Figure 1), but they have declined more for females (63.3%) than for males (48.8%). By contrast, for all NHANES periods examined combined, PFOS LSGM concentration increased with age regardless of sex, and the increase was more pronounced in females than in males (Figure 2). PFOS LSGM concentrations also increased with age, regardless of race; however, the increase was more pronounced for non-Hispanic blacks than for other race/ethnic groups. Regardless of age, Mexican Americans had PFOS LSGM concentrations significantly lower than non-Hispanic whites ($P < 0.01$), who, in turn, had significantly lower LSGM PFOS concentrations than non-Hispanic blacks—except that non-Hispanic whites and non-Hispanic blacks at 20−39 years of age had similar concentrations ($P = 0.93$).

Males also had higher PFOA LSGM concentrations than females, regardless of survey period (Figure 1). Among males

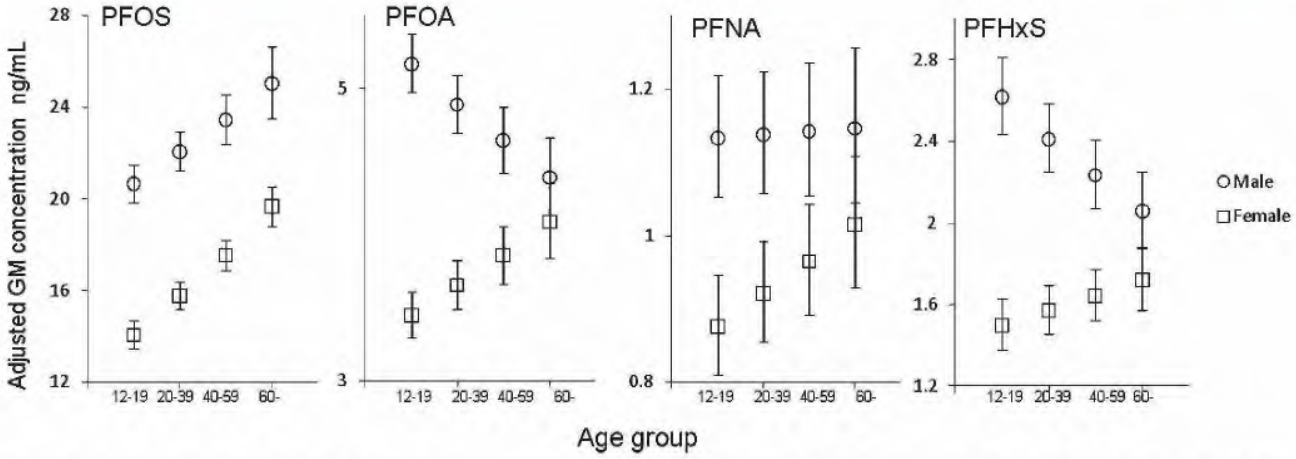

Figure 2. Age trend for the model adjusted geometric mean serum concentrations of several PFCs for all NHANES periods examined combined. The error bars represent the 95% confidence intervals.

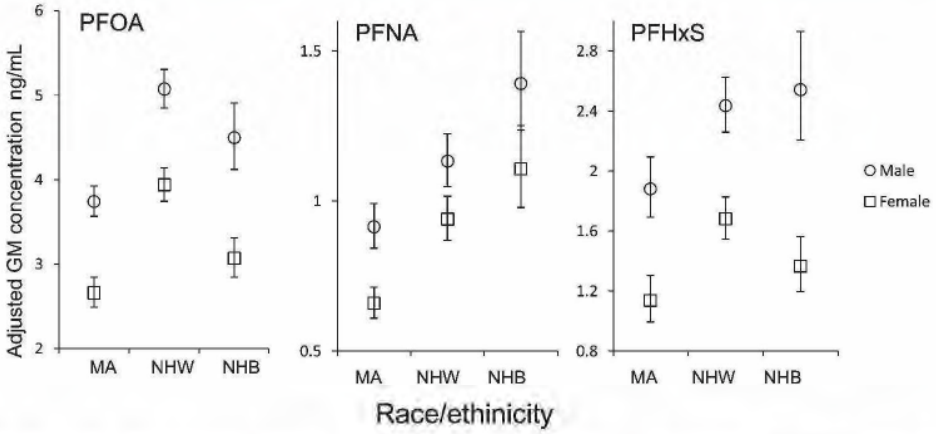

Figure 3. Sex trend for the model adjusted geometric mean serum concentrations of several PFCs by race for all NHANES periods examined combined. The error bars represent the 95% confidence intervals: MA (Mexican American), NHW (non-Hispanic white), NHB (non-Hispanic black).

and females, PFOA LSGM concentrations in 1999–2000 were significantly higher ($P < 0.01$) than during any other periods examined (SI Tables S13–S14). Among males, PFOA concentrations during 2003–2004 were similar to those concentrations in 2005–2006 ($P = 0.527$), but significantly lower ($P = 0.032$) than in 2007–2008; PFOA concentrations in 2005–2006 and 2007–2008 were not statistically different ($P = 0.259$). Among females, PFOA LSGM concentrations in the last three NHANES cycles were similar. Regardless of age, males had PFOA LSGM concentrations higher than females, but as age increased, PFOA concentrations decreased in males and increased in females (Figure 2 and SI Table S10). Also, PFOA LSGM concentrations were higher in males than in females regardless of race. Non-Hispanic whites had higher PFOA LSGM concentrations than non-Hispanic blacks ($P < 0.01$), who, in turn, had higher PFOA concentrations than Mexican Americans ($P < 0.01$), regardless of sex (Figure 3).

We observed a significant linear upward trend of PFNA LSGM concentrations across the survey periods examined, regardless of race. However, the increase was more pronounced in Mexican Americans than in non-Hispanic whites, and in the latter more than in non-Hispanic blacks (Figure 1). Mexican Americans had PFNA LSGM concentrations significantly lower than non-Hispanic whites ($P < 0.01$), who, in turn, had lower PFNA concentrations than non-Hispanic blacks ($P < 0.01$), regardless of survey period. However, in 2007–2008, non-Hispanic whites and non-Hispanic blacks had similar PFNA concentrations ($P = 0.06$) (SITables S13–S14). Of interest, PFNA LSGM concentrations in 2007–2008 are 3.6 times (Mexican Americans), 2.5 times (non-Hispanic whites), and 2 times (non-Hispanic blacks) higher than during 1999–2000. Males had PFNA LSGM concentrations significantly higher ($P < 0.01$) than females regardless of age and race (Figures 2–3). Also, PFNA LSGM concentrations increased with age regardless of sex, but the increase was more pronounced in females than in males. Similarly, PFNA LSGM increased as age increased regardless of race; however, the increase was most pronounced among non-Hispanic blacks (SI Figure S1). Regardless of age, Mexican Americans had PFNA LSGM concentrations significantly lower than non-Hispanic whites and non-Hispanic blacks ($P < 0.01$). Non-Hispanic blacks had significantly higher concentrations than non-Hispanic whites, but similar concentrations at 12–19 years of age ($P = 0.22$).

PFHxS LSGM concentrations showed a downward trend from 1999–2000 to 2005–2006, but in 2007–2008, LSGM concentrations were similar to those in 1999–2000, particularly

for males (Figure 1 and SI Table S14). Overall, males had significantly higher LSGM PFHxS concentrations than females ($P < 0.01$), regardless of survey period.

For the combined NHANES cycles, we found that males had higher LSGM concentrations of PFOS, PFOA, and PFHxS than females regardless of age. Furthermore, males had higher LSGM concentrations of PFOA, PFHxS, and PFNA than females regardless of race/ethnicity. Higher PFOS concentrations among males than among females have been reported before,[11,12,15,16,18] suggesting the possibility of sex-related differences in exposure. For PFOA and PFHxS, we observed similar trends in concentrations by sex regardless of age and race. Males had higher concentrations than females, but the concentrations among males decreased with age, while the opposite trend existed for females. By contrast, for PFOS and PFNA, concentrations tended to increase with age regardless of sex; for PFNA, this age-related increase was much more pronounced among females than among males. Interestingly, the differences in concentrations of PFOS, PFOA, PFNA, and PFHxS by sex were more pronounced in younger than in older people. These concentration trends may be related to sex-related differences in exposure to these PFCs even at an early age; they may also be related to physiological differences by sex, including menstruation, pregnancy, and/or lactation.[3]

We also observed differences in PFCs LSGM concentrations according to race; some of these differences were modulated by sex and age. Regardless of age, Mexican Americans had lower PFNA concentrations than non-Hispanic whites and non-Hispanic blacks. For PFHxS, non-Hispanic whites and non-Hispanic blacks had similar concentrations, and both were higher than for Mexican Americans; at older ages, however, concentrations were different only among Mexican Americans and non-Hispanic whites. Sex and racial differences may reflect variability in exposures related to lifestyle, diet,[3] use of products containing PFCs, physiology (e.g., elimination), or a combination of all of the above.

Since the changes in manufacturing practices of PFCs, serum concentrations of several PFCs have shown a downward trend.[11−13] In our analysis, PFOS LSGM concentrations showed a clear downward trend from survey period 1999−2000 to 2007−2008 regardless of sex, even though the trend was more pronounced in females than in males. For PFHxS, we observed a downward trend for both males and females from 1999−2000 to 2005−2006, but an increase in concentrations, much more pronounced among males than females, during 2007−2008 compared to previous survey years. Similarly, PFOA concentrations decreased from 1999−2000 to 2003−2004 both for males and females, but they appeared to increase for males from 2005 to 2008 or remain pretty much the same for females during the same time period. By contrast, regardless of race, PFNA concentrations showed a clearly increasing trend since 1999−2000.

The decrease in PFOS serum concentrations during the time interval encompassed since NHANES 1999−2000 until the most recent NHANES 2007−2008 agreed with the reported reduction in PFOS concentrations among U.S. Red Cross blood donors.[12] These reductions in concentrations in humans and also in wildlife had been related to the phase-out of PFOS and PFOS-related materials.[12,19] The observed PFHxS temporal trend is difficult to explain because production of PFHxS presumably stopped with the discontinued manufacture of PFOS-related materials, unless unknown sources of PFHxS in the environment still exist. However, these additional sources would have to affect males and females differently, which appears unlikely, or substantial differences by sex exist in the elimination half-life of PFHxS; such a sex differences may also be explained if PFHxS is still used in occupational settings.

Direct and indirect (e.g., polyfluoroalkyl phosphate diesters,[20] fluorotelomers,[21] perfluorinated phosphonic acids[22]) sources of PFOA still exist in the United States, even though since 1999 and up to 2004, global emissions of PFOA reportedly had decreased by more than half[2] and current producers have committed to reducing manufacturing emissions of PFOA and its salts and precursors.[7] Until 2002, production of PFOA involved electrochemical fluorination (ECF), which produced mixtures of linear and branched isomers, and telomerization, which produced only linear isomers.[1] When production of PFOS stopped in 2002, telomerization became the only process for manufacturing PFOA and other PFCs. We detected branched PFOA isomers in 96.9% of NHANES 1999−2000 participants, with a median (25th−95th percentiles) percentage of branched PFOA isomers (estimated as 100 times the ratio of concentrations of branched to total (branched plus linear) isomers) of 4.2% (2.7−9.9%), suggesting that ECF production contributed to PFOA exposure before 2002. By contrast, among NHANES 2007−2008 participants, we found no evidence of the presence of branched PFOA isomers; rather, we detected only the PFOA linear isomer. These findings suggest that telomerization products likely continue to contribute to PFOA exposure after the phase-out of ECF products.[21] However, because these data are rather limited, they require replication before it is possible to draw inferences from them.

Interestingly, we observed an upward trend, regardless of race/ethnicity, in PFNA concentrations since 1999−2000. PFNA and other perfluorocarboxylates (PFCAs) were present as reaction byproduct in ECF-based materials,[2] which are no longer produced in the United States. Therefore, the observed increase in PFNA concentrations may be related to the degradation of the volatile fluorotelomer alcohols.[21] Of note, although the PFC profiles in humans and wildlife generally differ, the human data agree with some other data suggesting that concentrations of PFNA and certain longer chain-length PFCAs show an upward trend in wildlife during the same time period.[23]

In summary, these NHANES 1999−2008 data are consistent with reduced population exposure to PFOS because of efforts of industry and government. For PFHxS, the data suggest reduced exposure from 1999 to 2006, but increased exposure during 2007−2008. The NHANES data also suggest that during 2003−2008, PFOA exposure, although significantly lower than in 1999−2000, has remained essentially unchanged while for PFNA, human exposure during 1999−2008 has shown an upward trend. These NHANES data also suggest that sociodemographic factors, which likely influence lifestyle choices, may contribute to PFCs exposure. However, predictors of PFCs serum concentrations have not been clearly identified and additional research is needed to identify the environmental sources that contribute to human exposure to PFCs. Also, we will continue to assess exposure to PFCs in the U.S. population through NHANES, an effort that will provide unique information on temporal trends of exposure to PFCs.

■ ASSOCIATED CONTENT

Supporting Information. Analytical method details (Table S1); serum concentrations of additional seven PFCs

(Tables S2–S8) and discussion about changes in their detection frequencies; regression model results (Tables S9–S12); LSGM concentrations of PFOS, PFOA, PFHxS, and PFNA and observed statistical significance values for their differences (Table S13); and age trend by race of LSGM concentrations of PFNA and PFHxS (Figure S1). This material is available free of charge via the Internet at http://pubs.acs.org.

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ACKNOWLEDGMENT

We thank Jack Reidy, Xavier Bryant, Jason Tully, Amal Wanigatunga, and Brian Basden for technical assistance. The authors have no competing financial interests to declare. Disclaimer: The findings and conclusions in this report are those of the authors and do not necessarily represent the views of the Centers for Disease Control and Prevention.

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EXHIBIT C-72

MR#350977

DuPont Haskell Global Centers
for Health and Environmental Sciences
1090 Elkton Road, P.O. Box 50
Newark, DE 19714-0050

January 8, 2013

Via Federal Express

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Document Processing Center (Mail Code 7407M)
Room 6428
Attention: 8(e) Coordinator
Office of Pollution Prevention and Toxics
U.S. Environmental Protection Agency
1201 Constitution Ave., NW
Washington, DC 20004

Dear 8(e) Coordinator:

8EHQ-06-16436/8EHQ-06-16478
2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)propionic acid, ammonium salt
CAS # 62037-80-3

This letter is to inform you of the preliminary results of a 2 year rat oral gavage study with the above referenced test substance. This test substance is subject to a Consent Order, P-08-509.

A 2-year oral gavage study was conducted in Crl:CD(SD) rats (80/sex/concentration) with the test substance at doses of 0, 0.1 (males only), 1, 50, and 500 (females only) mg/kg bw/day. The rats were evaluated for mortality, clinical signs, body weight and weight gain, food consumption, and food efficiency, and received an ophthalmology examination pretest and after 1 and 2 years of dosing. Ten rats/sex/dose were designated for evaluation of chronic toxicity. These rats were evaluated for clinical pathology at 3, 6, and 12 months, and for anatomic pathology (organ weights, gross and microscopic pathology) at the end of 12 months. The remaining rats (70 rats/sex/dose; main study rats) were dosed for up to 23 (females) or 24 (males) months. Females were sacrificed at week 100 due to poor overall survival, although survival was comparable among all dose groups. Clinical pathology (WBC differential counts) was evaluated at 12, 18, and 24 months in all surviving main study rats. All animals received a gross pathology evaluation at necropsy, and organ weights were collected in animals surviving to terminal sacrifice. Microscopic examination of tissues was conducted in animals that survived to scheduled sacrifice (12 month and end of study), and in all animals that died prior to scheduled sacrifice.

No test substance-related differences in survival or in clinical or ophthalmological signs were observed in any dose group. No adverse effects on overall body weight and nutritional parameters were observed in any dose group, although these parameters were transiently lower than control (statistically significant) in high-dose males (50 mg/kg/day) and females (500 mg/kg/day) over some weekly/biweekly intervals, particularly during the middle of the study. In 500 mg/kg/day females, the body weight over the first year of the study was statistically significantly lower than in control, although the difference was not statistically significant at the end of two years. Test substance-related, adverse or potentially adverse findings were observed in some clinical and anatomic pathology parameters in females at 500 mg/kg/day and in males at 50 mg/kg/day parameters, as discussed below.

Clinical pathology: The following statistically significant differences were considered adverse:

500 mg/kg/day (females only):

- ↓ red blood cell mass parameters (RBC, HGB, HCT, most time points), with ↑ MCV and reduced MCHC at the 12 month time point.
- ↑P (12 month), ↑ BUN (12 month), ↑A/G ratio (all time points), ↓globulin (all time points),

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- urine: ↑urine volume and pH, ↓specific gravity (6, 12 month)

50 mg/kg/day:

- ↑ALP (male all time points), ↑ALT (male 12 month), ↑albumin (male all time points), ↑A/G ratio (male all time points)

Anatomic pathology: Increases in the following microscopic pathology findings were considered adverse:

500 mg/kg/day (females):

- Liver: adenoma, hypertrophy (also ↑ at one year), degeneration and necrosis; ↑ liver weight (at one and two year)
- Kidney: papillary necrosis and edema, chronic progressive nephropathy (also ↑ at one year), dilated tubules,
- Stomach: non-glandular mucosal hyperplasia
- Tongue: mucosal hyperplasia/inflammation

50 mg/kg/day:

- Liver: ↑ liver weight (males at one year only), hypertrophy, degeneration and necrosis (also ↑ in males at one year), basophilic foci; (males only except hypertrophy)
- In males, marginal increases were observed in the following:
  - Pancreas: acinar cell tumors; equivocal acinar cell hyperplasia (both sexes)
  - Testes: interstitial cell tumors and hyperplasia

All other statistically significant changes in clinical and anatomic pathology parameters were considered spurious and/or nonadverse based on absence of a dose response, the transient occurrence of the finding, the minimal nature or direction of the change, and/or the lack of correlative changes in related parameters. These included:

500 mg/kg/day (females only)

- ↑ Cl (6 month), ↑albumin (3 month), ↓bilirubin (all time points), ↓total protein (3 month), ↓ cholesterol (6 month), ↓APTT (12 month)
- Uterus: stromal polyps (not significant by Fisher's exact test and within historical control range)
- Lung: histiocytosis (within historical control range)
- Adrenal: benign pheochromocytoma (not significant by Fisher's exact test, within historical control range and not associated with correlative increase in hyperplasia)

50 mg/kg/day:

- ↓red blood cell mass parameters (RBC, HGB, HCT) at all time points in males; ↓RBC in females (12 month)
- ↓APTT (12 month; female))
- ↑ Ca (male 12 month), ↑P (male 3 month), ↑A/G ratio (female 3 and 6 month), ↓globulin (female 6 month)
- Urine: ↓urine volume (male 12 month) and pH (male 6 and 12 month)

1 mg/kg/day:

- ↓HGB (female 3 month), ↑ALP (male 12 month), ↑ BUN (male 12 month), ↑albumin (male 12 month), ↑A/G ratio (male all time points), ↑Cl (female 6 month)
- Urine: ↑urine volume (male 12 month) and pH (male 6 and 12 month; female 6 month)

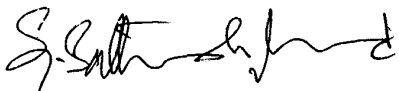
0.1 mg/kg/day (males only):

- ↑P (3 month)
- Urine: ↓urine volume and ↓ pH (both 12 month)

Under the conditions of this study, the no-observed-adverse-effect level (NOAEL) was considered to be 1 mg/kg/day in male and female rats. Test substance-related neoplastic changes were observed at the high dose (500 mg/kg/day in females; 50 mg/kg/day in males) and included hepatocellular tumors in females and, in males, equivocal increases in pancreatic acinar cell tumors and testicular interstitial cell tumors. These tumor findings are typical of those previously reported in rats following exposure to other PPARα agonists. Based on the high dose threshold for these tumor responses in this study, the lack of genotoxicity of the test material across a battery of *in vitro* and *in vivo* tests, and the known responses of the rat versus other species, including humans, to these PPARα-associated tumor responses, these tumor findings are not considered relevant for human risk assessment.

This information is submitted in accordance with current guidance issued by EPA indicating EPA's interpretation of Section 8(e) of the Toxic Substances Control Act or, where it is not clear that reporting criteria have been met, it is submitted as a precautionary measure and because it is information in which EPA may have an interest.

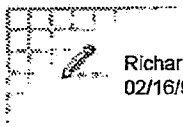
Sincerely,



S. Satheesh Anand, Ph.D., DABT
Senior Research Toxicologist

SSA/SAM: jhh
(302) 366-5314

EXHIBIT C-73



Richard E. Purdy
02/16/99 07:00 AM

To:
cc:
Subject: Request for Analysis of data essential for estimation of environmental harm

As I recall, you as a committee found that my submission of December 4, 1998 was not 8e reportable because the data used was speculative. You said that the risk assessment should be based on laboratory analysis of fishmeal and rodent chow. And I assumed that meant that the 3M environmental laboratory was directed to do those analysis on the samples they had. I have been provided a verbal report on the concentration in Purina Certified Rodent Chow, but not for fish meal. It has been two months since the request was made. I have asked the analyst, who says it has not been made a priority for her. I have asked Bill Reagen and Dale Bacon several times to direct these samples to be analyzed. The samples still have not been analyzed. I have used an approximate value given to me some time back by Kris Hansen. She says this is a range finding number and has not been validated, so she will not stand behind it. I have found that her range finding numbers have been close to the eventual validated value in the past. I have used this value in a second generation risk assessment which is attached.

I view the avoidance of analysis to be irresponsible. Perfluorooctane sulfonate (PFOS) pollution of the environment the worst since PCB. I am suspicious that it is worse. Toxicology agrees with me that the risk of ecological harm is far more likely than harm to humans. There appears to be a corporate policy to avoid evaluating the ecological risk PFOS, the most persistent pesticide ever registered. It starts back in 1983 when the Ecological Science and Assessment group proposed testing to the division. The proposal evaluated the data and the data needs. We still have not collected any of the data. John Geisy of Michigan state also provided 3M with a report on what should be done. Robert Howell worked for years to get 3M to test PFOS. He left 3M frustrated. I have been working on the issue for about 10 months now and have made little progress because of road blocks. I have requested to view customer data on a closely related chemical for 9 months and 3M has only recently set up that visit. The original 3M Exposure Team recommended the analysis of fish eating bird livers and obtain samples for the U.S. Fish and Wildlife Service. These samples have not been analyzed. I asked regularly for these samples to be completed. Likewise I continue to ask to the analysis of bird and fish tissues from a feeding study completed about two years ago. Again I meet passive resistance. For the last 6 months I have been asking that 3M hire consultants with expertise in ecological risk assessment. I have identified the best in the field. I have set up a secrecy agreement with one group so we can have them in for our evaluation. There has been movement from 3M management. I view the passive resistance of my immediate management to originate from committees that direct the companies efforts on the issue.

Attached to this memo are: 1.) The pioneer risk assessment submitted to Georgean Adams on December 4, 1998. This is provided for reference.
2.) A review of Latham and Watkins' evaluation of that risk assessment as reportable under TSCA 8e.
3.) The first iteration of the pioneer risk assessment. This uses the little data provided. All sections have been modified and expanded to evaluate more food chains. 4.) My resume is attached for those of you who are not familiar with my credentials.

EXHIBIT C-74

28 March 1999

To: 3M

I resign my position as Environmental Specialist effective 6 April 1999. My resignation is prompted by my profound disappointment in 3M's handling of the environmental risks associated with the manufacture and use of perfluorinated sulfonates (PFOS)(CAS# 29081-56-9) and its precursors, such as ethyl FOSE alcohol (CAS #1691-99-2) and methyl FOSE alcohol (CAS #24448-09-7).

Perfluorooctansefulfonate is the most insidious pollutant since PCB. It is probably more damaging than PCB because it does not degrade, whereas PCB does; it is more toxic to wildlife; and its sink in the environment appears to be biota and not soil and sediment, as is the case with PCB.

I have worked within the system to learn more about this chemical and to make the company aware of the dangers associated with its continued use. But I have continually met roadblocks, delays, and indecision. For weeks on end I have received assurances that my samples would be analyzed soon--never to see results. There are always excuses and little is accomplished. I can illustrate with several examples.

- For more than twenty years 3M's ecotoxicologists have urged the company to allow testing to perform an ecological risk assessment on PFOS and similar chemicals. Since I have been assigned to the problem a year ago, the company has continued its hesitancy.
- Over a period of seven months I made frequent requests that ecological risk consultants be hired to help me plan toxicity testing, environmental sampling, chemical fate studies, and ecological risk procedure. I still have not received authorization even to bring people in to interview.
- I requested, very frequently, over a nine-month period, a sample of chemical to send out for fate property and ecotoxicity testing. Finally I was provided with one that apparently the division had had all along.
- I put together a pioneer risk assessment on PFOS that indicated a greater than 100% probability of harm to sea mammals, based on preliminary data on the concentration of PFOS in menhaden fish meal. The 8e committee told me that they would like to reconsider the assessment after we had a validated value for fishmeal. That analysis was given high priority by the committee. After three months the analysis is still not done--not because there were technical problems, but because management did not actually give the analysis high priority.
- 3M submitted a TSCA 8e last May. There is tremendous concern within EPA, the country, and the world about persistent bioaccumulative chemicals such as PFOS. Just before that submission we found PFOS in the blood of eaglets--eaglets still young enough that their only food consisted of fish caught in remote lakes by their parents. This finding indicates a widespread environmental contamination and food chain transfer and probable bioaccumulation and bio-magnification. This is a very significant finding that the 8e reporting rule was created to collect. 3M chose to

Exhibit
1001
State of Minnesota v. 3M Co.,
Court File No. 27-CV-10-28862

report simply that PFOS had been found in the blood of animals, which is true but omits the most significant information.

- One of our customers, Griffin, has data on some of our chemicals. They developed this data for pesticide registration purposes. I started regularly asking for permission to visit Griffin and view the data last May. Their data can help us plan our studies of similar chemicals. It can also indicate if there is an unforeseen risk to certain biota or via certain exposure pathways. It was ten months before I was allowed to visit Griffin, at which time I did not get to see the data. I have to return another time to see it.
- 3M waited too long to tell customers about the widespread dispersal of PFOS in people and the environment. We knew before May of 1998, yet 3M did not start telling customers until January of 1999. I felt guilty about this and told customers I personally knew earlier. Still, it was not as early as it should have been. I kept waiting for 3M to do its duty, as I was continually assured that it would. Some of the customers have done risk assessments on the PFOS precursor they use. They assume there is not a background in the environment and in wildlife. Since there is a background, their risk assessments are inaccurate. Thus they can make inappropriate business decisions and not realize that their use of PFOS precursors contributes to an aggregate risk.
- 3M continues to make and sell these chemicals, though the company knows of an ecological risk assessment I did that indicates there is a better than 100% probability that perfluorooctansulfonate is biomagnifying in the food chain and harming sea mammals. This chemical is more stable than many rocks. And the chemicals the company is considering for replacement are just as stable and biologically available. The risk assessment I performed was simple, and not worst case. If worst case is used, the probability of harm exceeds 100,000%.

- 3M told those of us working on the fluorochemical project not to write down our thoughts or have email discussions on issues because of how our speculations could be viewed in a legal discovery process. This has stymied intellectual development on the issue, and stifled discussion on the serious ethical implications of decisions.

I have worked to the best of my ability within the system to see that the right actions are taken on behalf of the environment. At almost every step, I have been assured that action will be taken—yet I see slow or no results. I am told the company is concerned, but their actions speak to different concerns than mine. I can no longer participate in the process that 3M has established for the management of PFOS and precursors. For me it is unethical to be concerned with markets, legal defensibility and image over environmental safety.

Sincerely,

Rich Purdy

EXHIBIT C-75

Date: Sat, 30 Aug 2003 07:17:34 -0500
>To: Charlie Auer, steve.johnson@epamail.epa.gov
>From: rich purdy <rpurdy@pressenter.com>
>Subject: postponing of risk assessment.

>
>I heard that EPA is postponing the final risk assessment for PFOA and Science Advisory Board meeting from this fall until next spring because industry is doing another study. If this is true, it is outrageous. PFOA is obviously causing harm now as part of the perfluorinated acid family. Your draft risk assessment on PFOA shows that it presents too much risk by itself, even without considering additivity with other family members, which means the risk is worse than your assessment on just PFOA indicates.
>
> Postponing is a normal industry tactic. They continue with multi billion-dollar sales per year and millions in profit each day. The current managers at the corporations push off the falling of the axe until after they retire or leave. There was a recent court decision against Monsanto. The monetary level was about 700 million dollars. By my calculations that is less than the yearly profit for manufacturers of telomers and those who use PFOA. The longer they postpone action, they longer they make profits and set them aside for settlements. Dupont's philosophy can be seen in the May 22, 1984 memo from J.A. Schmid to T.M. Kemp, T.L Schrenk and R.E. Putnam in which a meeting on the recent detection of C-8 in Little Hocking, OH, and Lubeck, WV, water systems was discussed. Appling the logic in that memo to today's issues: Since duPont has polluted the environment for 50-odd years, they cannot become any more liable. So what's a few more years of pollution. Especially, if they are pulling in hundreds of millions in profits each year.
>
>We see in the PFOA Environmental Consent Agreement meetings the same dragging of feet. Industry refuses to discuss looking at blood levels, soil levels, levels in fish, levels in mammals, levels in vegetation, levels of isomers or homologs that indicate source of pollution, etc. The experts in the room say that is what is needed, and industry's managers and lawyers say no. By the way why are there 5 managers and lawyers to every one industry scientist at these "technical" workgroup meetings?
>
>EPA is not doing its job. Postponement means more children with birth defects, more developing asthma, more developing early onset diabetes, and who knows what else. PFOA and its family of chemicals are causing more health problems than any other industrial chemical. And it lasts forever. We do not even know yet if equilibrium has been reached. We are likely to continue accumulating higher and higher levels in our blood and livers for years and maybe decades even if all production and release were to halt tomorrow.
>
>You have to stop this crime from continuing. Regulate now and stop allowing Industry to postpone the day of judgment until after you retire, too. You told 3M your concerns, they realized the error of their ways and halted production of PFOA and other members of the family. You have shown duPont, Atofina, Daikin, Ashi Glass, Ciba, Mitsui,

Clariant and the other current producers even more damning information. They obviously are not smart enough to understand or else they are immoral. Otherwise they too would have halted production. Jawboning or painting them into a corner is not working. They are obviously in the polluters' stall mode. They will not take any action that does not help them stall. In their mind any action on their part might bring lawsuits. You have to act.
>
>Respectfully,
>Rich Purdy, PhD
>

EXHIBIT C-76

From: Jenny Liu

Sent: Friday, July 23, 2010 1:34 AM

To: Andrew S Hartten; David W Boothe; Fred C Dawson; Gregory W Smith; holtrf1@comcast.net; James R Hoover; John M Schofield; L William Buxton; Leo J Hyde; michaelmccabe1@earthlink.net; Minori Hagiwara; Nancy S Selzer; Paul J DiAntonio; Robert C Buck; Robert W Rickard; Ronald P Bock; T C Feng; Andrea V Malinowski; Eric A van_Wely; Kathleen A Shelton; Yolande Peeters; Jorge Dieguez; Akito Abe; Gloria Xu; John Qiang Zhao; Chun Ku Chen; Kenny Jeng; Tency N L Du; Carol Ke Wen Chen; Barry M Granger; Linda A Strachan; Gregory W Smith; Michael S Parr; Chris Caldwell; Rick M Deadwyler; Jeff Fritz; cindy.goldstein@pioneer.com; billi.hunt@pioneer.com; sarah.thorn@pioneer.com; Warren E Mayberry; erin.spencer@pioneer.com; thomas.r.jacob@gmail.com

Cc: Janet E Smith; Dawn R Werry; Janice L Connell; John W Moriarty; Kirsten E Myers; Laura A Korte; Martha L Rees; Mary Erin Mariani; Pascal Ferrandez; Paul D Berg; Paul N Costello; Wayne M Lednar; Thomas H Samples; Frenk Hulsebosch; Stephen Rahaim; Maria S Angelo; Patricia McGee; Ann K Masse; Diane Norvell

Subject: PFOA Overview

Attach: PFOA Overview 2010-07-22.pdf

Global R/G team and GA managers,

Attached is a "backgrounder" on PFOA that is intended as a leave-behind for use with regulatory and government audiences. This would generally be handed out in the context of a face-to-face meeting. It is 2 double-sided pages in length. Many thanks to Janet for her help on this. Please call me if you have any questions regarding its content or use.

Best regards,
Jenny

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PFOA Overview

PFOA (perfluorooctanoic acid) is a persistent chemical present at very low levels in the environment and the blood of the general population. A number of global manufacturers, including DuPont, make and use PFOA. PFOA is a polymerization processing aid used to produce some high-performance fluoropolymer materials. While PFOA is not used to make fluorotelomers, it is found at very low trace levels in some fluorotelomer products as a byproduct of their synthesis.

Current DuPont fluoropolymer and fluorotelomer products which may contain low levels of PFOA are safe for their intended uses and offer significant benefits. Our studies indicate that any PFOA present in consumer products made with DuPont materials is at extremely low trace levels. Use of these products does not result in measurable quantities of PFOA in the blood of consumers. More information is available at www.pfoa.dupont.com.

DuPont Phase-out Commitment: In response to questions about PFOA in the blood of the general population, and customer interest in product alternatives, DuPont has made a commitment to no longer make, buy or use PFOA by 2015, or earlier if possible. We have developed new products and processes that are more environmentally sustainable.

EPA 2010/15 PFOA Stewardship Program: DuPont is one of eight major manufacturers participating in the U.S. Environmental Protection Agency (EPA) 2010/15 PFOA Stewardship Program, which was announced in January 2006. DuPont is on track to meet or exceed the 2010 program goal of 95% reduction in emissions and product content. In fact, we have reduced our worldwide manufacturing PFOA emissions by 98%. The U.S. Centers for Disease Control and Prevention (CDC) has reported a 25% reduction in PFOA serum concentrations. EPA attributes these reductions to industry and Agency efforts.

Environment: PFOA has been made and used for more than 50 years. The total amount of PFOA made and used is not large compared to most industrial chemicals. In recent decades, analytical methods have improved so that they can now detect substances at extremely low trace levels (parts per trillion). Data generated through the use of advanced analytical methods show that very low levels of PFOA are present and fairly widespread in the environment.

Human Health: Based on extensive health and toxicological studies, DuPont believes that PFOA exposure does not pose a health risk to the general public. Human studies have evaluated many health endpoints across a wide range of exposed populations. While some associations have been reported, no human health effects are known to be caused by PFOA. A considerable number of human health studies are ongoing, and results will be available over the next several years.

PFOA has been extensively studied in an occupational setting where potential exposure can be significantly higher than in the general population. DuPont continues to take steps to better understand and minimize exposure to PFOA in our global facilities. Our comprehensive industrial hygiene program has helped our sites improve their industrial hygiene practices and engineering controls.

Fluoropolymers

Fluoropolymers such as polytetrafluoroethylene (PTFE) are high molecular weight polymers with inherent properties that cannot be achieved with any other known substances. They have high thermal stability, low friction, and excellent electrical insulation properties, and are non-flammable and resistant to chemical attack. Certain fluoropolymers are manufactured using PFOA as a processing aid. PFOA is neither reacted with nor incorporated into the fluoropolymer. A variety of processes, including high heat treatment, are used to reduce PFOA content to trace levels in final products.

Fluoropolymer-based products play a critical role in many applications. These range from providing cable and internet service, generating clean and renewable energy, and manufacturing more efficient and reliable vehicles, to designing safe and high performance buildings and aircraft, and building lightweight and affordable laptops, cell phones, media players and home theaters.

New DuPont™ GenX Technology

DuPont has developed patented technology that enables us to make high-performance fluoropolymer resins without the use of PFOA. This includes a new-generation processing aid with a favorable toxicological profile and very rapid bioelimination, combined with unique environmental exposure control technologies that reduce the potential for environmental release and exposure.

The new processing aid is chemically stable and, if released, would be environmentally persistent. To address this, DuPont has established a GenX exposure control strategy with the goal of containing the new processing aid within the manufacturing site and minimizing worker exposures. We expect to operate with 99 percent or greater overall environmental control efficiency with the GenX processing aid, and to demonstrate that extractable residual processing aid content in fluoropolymer resins is less than 200 parts per billion (ppb). This information is available at www.genx.dupont.com.

We began in 2009 to convert customers to the use of fluoropolymers made with our alternative technology, which enables DuPont and our customers to continue to provide high performance fluoropolymer-based products that meet exacting end-use requirements.

Fluorotelomers

PFOA is not used to make fluorotelomers. However, it may be found at very low trace levels in some fluorotelomer products as a byproduct of their synthesis.

Fluorotelomers are raw materials used to produce surface protection products, including repellents and surfactants, for a wide range of applications in home furnishings, textiles, paper, fire-fighting foam, nonwovens, coatings, and stone and tile protection. The unique performance of fluorotelomer products brings consumers many benefits, which include ease of care, reduced maintenance, and extended life for a broad range of articles used every day.

New Capstone® Short-Chain Repellent and Surfactant Products

DuPont has developed a new line of surface protection products based on sustainable short-chain technology (six or less fluorinated carbons) that deliver superior performance, supported by extensive environmental, health and safety testing. Capstone® repellent and surfactant products are based on short-chains that cannot break down into PFOA or PFOS (perfluorooctane sulfonate) in the environment, and they are manufactured using patented technology to minimize the presence of residual unreacted raw materials and by-products.

Extensive studies show that DuPont™ Capstone® repellent and surfactant products, raw materials such as short-chain alcohol, and potential degradation products including perfluorohexanoic acid (PFHxA) have a favorable environmental, health and safety profile, rapid bio-elimination, and are not bioaccumulative. This knowledge foundation is a comprehensive body of environmental, health and safety data show that Capstone® repellent and surfactant products are safe for workers, consumers and the environment when used as intended. This information is available at www.capstone.dupont.com.

PFHxA, a degradation product that may be formed at low concentrations, is a persistent substance in the environment. However, PFHxA has rapid bio-elimination, low toxicity, and it is not bioaccumulative. Published, peer-reviewed scientific studies have concluded that perfluorinated carboxylic acids (PFCAs) with less than eight total carbons, including PFHxA, are not bioaccumulative according to global regulatory criteria.

DuPont™ Capstone® repellent and surfactant products are used commercially as repellent s in home furnishings, paper packaging, textiles, stone and tile, and leather end uses and used as surfactants is fire-fighting foams and coatings. Capstone® repellent and surfactant products perform as well as, or better than, the products they replace.

Regulatory Activities - PFOA

- There are no restrictions on manufacture or use of PFOA and its salts anywhere worldwide.
- No authoritative body[1] has classified PFOA as a human carcinogen or human reproductive toxicant.
- PFOA is not a P-B-T (Persistent, Bioaccumulative and Toxic) compound nor does it meet the REACH vPvB (very Persistent, very Bioaccumulative) criteria. Although PFOA is environmentally persistent and moderately toxic, it is not bioaccumulative according to global regulatory criteria.
- Drinking water values have been established in the US and other countries to establish levels below which consumption is presumed safe, some for lifetime exposure.
- Tolerable Daily Intake levels have been established by the UK and the European Food Safety Authority.
- These regulatory positions and decisions have been informed by a considerable body of science on PFOA that numbers more than 300 toxicology and health related studies and a significant body of human epidemiology data.

22 July 2010

[1] as defined in California's Proposition 65 listing criteria